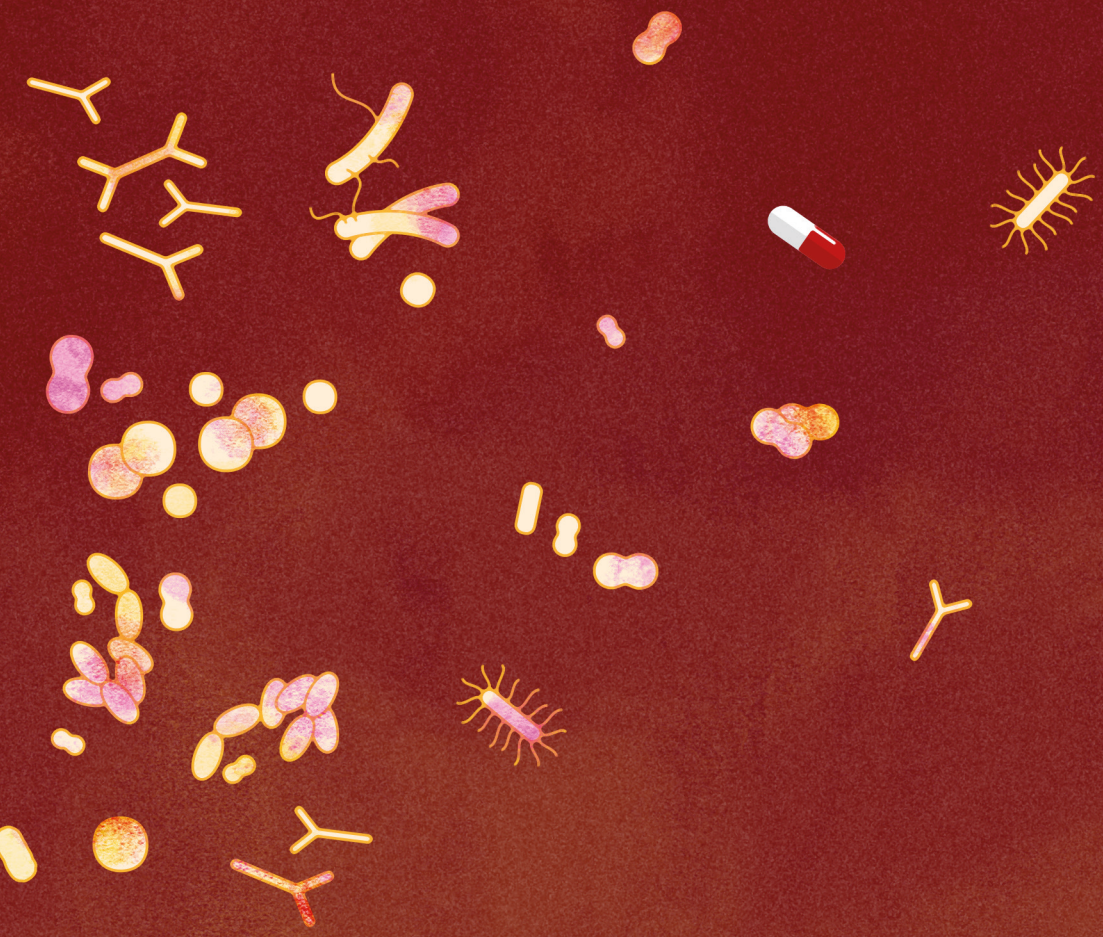
the epidemiology of infections with Clostridioides difficile and multidrug-resistant bacteria, and faecal microbiota transplantation as an intervention strategy

Vendrik, K.E.W.

Chapter 9

Summary



This thesis is divided into three parts. The first part describes the epidemiology of infections with *C. difficile,* meticillin-resistant *Staphylococcus aureus* and colistin-resistant Enterobacterales. An important treatment strategy for recurrent *C. difficile* infections is restoring the disturbed gut microbiota by FMT. The second part discusses the risk of transmission of pathogenic and/or multidrug-resistant bacteria via FMT and procedures to prevent this. Apart from recurrent *C. difficile* infections, several new potential indications of FMT are being explored. In the third part of this thesis, FMT is explored as a potential new treatment strategy for several neurological disorders, with a main focus on Parkinson's disease.

Part 1: The epidemiology of infections with *C. difficile* and multidrug-resistant bacteria

The epidemiology of CDI in the Netherlands has changed over the years. The hypervirulent ribotype RT027 caused worldwide major outbreaks in hospitals at the start of this century. In 2005 several major outbreaks were also notified in the Netherlands. This led to the foundation of the Dutch National Reference Laboratory for *C. difficile,* and the start of the Dutch sentinel CDI surveillance in 2009. This laboratory also offers ad hoc typing service for all Dutch microbiology laboratories for isolates from patients with severe disease or isolates from a suspected outbreak. The sentinel surveillance programme monitors the incidence and characteristics of CDI in the Netherlands and (new) emerging strains of *C. difficile*. Since the start of the Reference laboratory and the implemented measures to halt the spread of *C. difficile*, the incidence of this ribotype has decreased. Between May 2019 and January 2021 only 0.2% of *C. difficile* isolates had RT027. Simultaneously, the percentage of CDI patients in hospitals with severe disease has gradually been decreasing from 28% at the start of the Dutch CDI surveillance in 2009 to 16% in 2018-2019.[1] Since the end of 2019, the world has been heavily occupied with the coronavirus disease 2019 (COVID-19) pandemic, caused by the SARS-CoV-2 virus, and its consequences for the hospitals. **Chapter 2** describes a retrospective sentinel surveillance study on the epidemiology of CDI patients in hospitals during the COVID-19 pandemic for which we used data of the Dutch *C. difficile* surveillance. We found that the annual CDI incidence rate in 2020 was lower compared to the five previous years. During the second COVID-19 wave, the percentage of CDI patients with severe CDI was higher compared to previous years (26% vs 18% in 2015-2019). After adjustment for delayed *C. difficile* diagnostics (≥8 days from start symptoms), the increase disappeared. Delayed *C. difficile* diagnostics was more common during the second wave, but only for community-onset CDI (CO-CDI). This study showed that a higher percentage of severe CDI cases was observed during the second COVID-19 wave. This may partially be caused by delayed diagnostics, potentially

due to decreased visits of patients to a physician and restricted hospital referral for CO-CDI patients.

Another threatening Gram-positive bacterium is meticillin-resistant *Staphylococcus aureus* (MRSA). MRSA is a well-known nosocomially transmitted pathogen, but is also regularly reported in the community. **Chapter 3** discusses a community outbreak of impetigo by a MRSA strain, resistant to fusidic acid (the first-line treatment), in the eastern part of the Netherlands in July until September 2019. In total, 49 impetigo cases and 8 carrier cases were identified, including 42 children. Pharmacy prescription data for topical mupirocin and fusidic acid and questionnaires among general practitioners suggested the outbreak size may have been underestimated. The outbreak isolates were identified by the Dutch MRSA surveillance as MLVA-type MT4627 and ST121, previously reported only once in 2014. Next-generation sequencing revealed they contained a fusidic acid resistance gene (*fusC*), exfoliative toxin genes (*eta* and *etb*) and an epidermal cell differentiation inhibitor (*edinC)*. Whole-genome multi-locus sequence typing revealed genetic clustering of all 19 sequenced isolates from the outbreak region and three isolates from the north-western region. The allelic distances between these Dutch isolates and international isolates from the NCBI database were high. Due to collaboration between general practitioners, municipal health services, medical microbiologists and epidemiologists, the outbreak was halted in October 2019. However, since the end of 2020, a substantial number of new cases with impetigo due to MRSA MT4627 are being found in the south-western part of the country, mostly in children and small family clusters in the community. This indicates that the spread of this MRSA is continuing at a slow pace in the community in other regions. This outbreak shows the appearance of community-onset MRSA strains with drug resistance and virulence factors in a country with a low prevalence of antimicrobial resistance.

Among Gram-negative bacteria, ESBL- and carbapenemase-producing bacteria are particularly of concern. Furthermore, colistin-resistant bacteria are of serious concern since colistin is a last-resort treatment option against these MDRO. However, little is known about the incidence and epidemiology of colistin-resistant bacteria in the Netherlands. **Chapter 4** reports on a prospective matched case-control study on the genomic epidemiology of colistin-resistant Enterobacterales from Dutch patients. In a period of six months, 22 Dutch laboratories collected 72 colistin-resistant *E. coli* or *K. pneumoniae* (COLR-EK) isolates (75% *E. coli* and 25% *K. pneumoniae*) that met de inclusion criteria of the study. Patients with infection or colonisation with these COLR-EK isolates were compared to patients with colistin-susceptible *E. coli* or *K. pneumoniae* (COLS-EK) isolates and these were matched for patient location, material of origin and bacterial species. The use of colistin in the previous six months was identified

as risk factor for colistin-resistance. Colistin was used for selective decontamination of the digestive tract (SDD) or selective oropharyngeal decontamination (SOD) in all cases that had used colistin. SDD/SOD is in the Netherlands provided to vulnerable patients with a haematological disease or patients on the intensive care department to prevent infections. Of COLR-EK isolates, five contained *mcr-1* and two *mcr-9*. One isolate lost *mcr-9* after repeated sub-culturing, but retained colistin resistance indicating that *mcr-9* was not encoding phenotypically expressed colistin resistance. Among 46 sequenced COLR-EK isolates, genetic diversity was large and 19 (41.3%) isolates had chromosomal mutations potentially associated with colistin resistance. This study shows that colistin resistance is present but uncommon in the Netherlands and caused by the plasmid-mediated *mcr* gene in only a minority of COLR-EK isolates. In order to obtain insight into the epidemiology of colistin resistance, it is important that laboratories use reliable tests. More than half of the laboratories tested for colistin resistance using automated systems, which are less reliable than the gold-standard broth-microdilution, and only 53% screened all Enterobacterales for colistin resistance.

Part 2: Infectious adverse events of faecal microbiota transplantation

FMT is a very effective and approved treatment for recurrent CDI.[2-6]. In these patients, FMT is considered a rather safe treatment.[7] Common adverse events are gastrointestinal symptoms, such as diarrhoea and abdominal pain. Serious adverse events such as aspiration pneumonia, gastrointestinal haemorrhage or death, have also been described, but occur in no more than 5% of patients. Reports have been published on patients that became ill after received faecal suspensions containing MDRO or Shiga toxin-producing *E. coli*.[8,9] . This underlines that rigorous screening of faeces donors is important to prevent infectious complications. **Chapter 5** reports on a retrospective cohort study in which we examined whether the applied screening procedure of the Netherlands Donor Faeces Bank (NDFB) is sufficient to prevent the presence of MDRO in faecal suspensions approved for use in FMT. This procedure is similar to screening procedures of most other stool banks worldwide and includes periodic screening of donor faeces on potential pathogens and MDRO every two to three months with a quarantine period and extra screening on MDRO after travel abroad. Among 170 approved faecal suspensions that were collected in the quarantine period and that were not individually tested, none contained an MDRO. Furthermore, the percentage of healthy faeces donors that are colonised with MDRO in their faeces was assessed. Among initial screenings, six of 66 tested individuals were MDRO-positive (9%). The percentage of ESBL-producing *E. coli* among initial screenings was comparable to the percentages described in previous studies with Dutch healthy

subjects. Of 16 active donors, four (25%) became MDRO-positive at some time point during donation activities in a period of almost five years. An ESBL-producing *E. coli* was found in 14 of 19 (74%) detected MDRO among all screening results. This study emphasizes that healthy faeces donors are at risk to become colonised with MDRO at some point during donation activities and that periodic screening, as currently performed by the NDFB, is sufficient to prevent transmission of MDRO to FMT-recipients.

FMT is being investigated as a treatment strategy for several other indications including recurrent infections with MDRO. **Chapter 6** describes a case report of a 16-years-old female with underlying kidney disease that received FMT because of persistent intestinal colonisation with a multidrug-resistant *K. pneumonia*. This MDRO caused recurrent complicated UTIs in the patient. Unfortunately, the FMT resulted in transmission of an antibiotic susceptible *E. coli* strain with uropathogenic properties, causing UTIs in the recipient. The uropathogenic *E. coli* could be cultured from donor faeces and whole genome sequencing confirmed donor to recipient transmission. Unfortunately, the multidrug-resistant *K. pneumoniae* also caused a UTI four months after FMT. This study shows that potentially pathogenic micro-organisms that cause no disease in the donor can lead to an infection after transfer to vulnerable patients.

Part 3: Exploring faecal microbiota transplantation as treatment for various neurological disorders

FMT is also under investigation as a potential treatment strategy for several neurological disorders. **Chapter 7** provides a review of the potential new indications for FMT among neurological disorders. Clinical trials with FMT, mostly without control group and all without blinding, have been performed in patients with autism spectrum disorder (ASD). They showed beneficial effects on neurological symptoms.[10-12] For Parkinson's disease and multiple sclerosis, there are several convincing animal studies, that suggest that FMT may have a positive effect in humans, which is supported by some human case reports. For epilepsy, diabetic neuropathy, stroke, Tourette syndrome, Alzheimer's disease and Guillain-Barré syndrome, there are less (convincing) animal studies and/or case reports that suggest beneficial effects of FMT. After this review, results of more recent trials have become available. For ASD, an open-label study[13] without an ASD-control group suggested a beneficial effect of FMT on behavioural and gastrointestinal symptoms. For multiple sclerosis, a recent small non-controlled trial[14] suggested that FMT is safe and tolerable in this patient category and that intestinal permeability improves after FMT. For Tourette syndrome, results of a small non-controlled trial[15] suggested that FMT is safe in this patient category and it improved tic severity in four of five patients. Several trials with

FMT as treatment for the above mentioned neurological disorders are planned or ongoing, as well as for amyotrophic lateral sclerosis.[16] For example, seven studies with FMT/faecal microbiota products were registered on ClinicalTrials.gov for Parkinson's disease on the 26th of February 2023. Our review shows that FMT may be a promising treatment option for several neurological disorders. However, available evidence is still scanty and mostly restricted to case reports and/or limited numbers of animal studies. The data of our review were incorporated in a successful grant application of 40,000 euro from the Parkinson patients association for a pilot study with FMT for patients with Parkinson's disease.

Chapter 8 describes a study protocol for a single center, self-controlled, interventional, safety and feasibility donor-FMT pilot study in 16 PD patients. Clinical tests will be performed at several timepoints and faeces and blood will be collected. The results of several timepoints post-FMT will be compared to those of two timepoints pre-FMT. The primary objectives are to assess feasibility and safety of FMT in PD patients. Secondary objectives are to explore whether FMT leads to alterations of motor complications and PD symptoms in the short term, determine alterations in gut microbiota composition and donor-recipient microbiota similarities and their association with PD symptoms and motor complications, assess the ease of the study protocol and examine FMT-related adverse events in PD patients.

References

1. Vendrik K.E.W., et al. Thirteenth Annual Report of the National Reference Laboratory for Clostridioides difficile and results of the sentinel surveillance May 2018 - May 2019. Available from: https://www.rivm.nl/documenten/annual-report-c-difficile-reference-laboratory-may-2018-may-2019 (2019).
2. van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. *The New England journal of medicine* 2013; **368**(5): 407-15.
3. Cammarota G, Masucci L, Ianiro G, et al. Randomised clinical trial: faecal microbiota transplantation by colonoscopy vs. vancomycin for the treatment of recurrent Clostridium difficile infection. *Alimentary pharmacology & therapeutics* 2015; **41**(9): 835-43.
4. Kelly CR, Khoruts A, Staley C, et al. Effect of Fecal Microbiota Transplantation on Recurrence in Multiply Recurrent Clostridium difficile Infection: A Randomized Trial. *Annals of internal medicine* 2016; **165**(9): 609-16.
5. McDonald LC, Gerding DN, Johnson S, et al. Clinical Practice Guidelines for Clostridium difficile Infection in Adults and Children: 2017 Update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2018; **66**(7): 987-94.
6. Debast SB, Bauer MP, Kuijper EJ. European Society of Clinical Microbiology and Infectious Diseases: update of the treatment guidance document for Clostridium difficile infection. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2014; **20 Suppl 2**: 1-26.
7. Quraishi MN, Widlak M, Bhala N, et al. Systematic review with meta-analysis: the efficacy of faecal microbiota transplantation for the treatment of recurrent and refractory Clostridium difficile infection. *Alimentary pharmacology & therapeutics* 2017; **46**(5): 479-93.
8. DeFilipp Z, Bloom PP, Torres Soto M, et al. Drug-Resistant E. coli Bacteremia Transmitted by Fecal Microbiota Transplant. *The New England journal of medicine* 2019.
9. Zellmer C, Sater MRA, Huntley MH, Osman M, Olesen SW, Ramakrishna B. Shiga Toxin-Producing Escherichia coli Transmission via Fecal Microbiota Transplant. *Clinical Infectious Diseases* 2021; **72**(11): E876-E80.
10. Kang DW, Adams JB, Gregory AC, et al. Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. *Microbiome* 2017; **5**(1): 10.
11. Kang DW, Adams JB, Coleman DM, et al. Long-term benefit of Microbiota Transfer Therapy on autism symptoms and gut microbiota. *Scientific reports* 2019; **9**(1): 5821.
12. Zhao H, Gao X, Xi L, et al. Fecal microbiota transplantation for children with autism spectrum disorder. In: Wallace MB, editor. DDW 2019 ASGE Program and Abstracts; 2019 May 18-21; San Diego, CA. Gastrointestinal Endoscopy (2019). p. AB512–AB513. 2019.
13. Li N, Chen H, Cheng Y, et al. Fecal Microbiota Transplantation Relieves Gastrointestinal and Autism Symptoms by Improving the Gut Microbiota in an Open-Label Study. *Frontiers in cellular and infection microbiology* 2021; **11**: 759435.

14. Al KF, Craven LJ, Gibbons S, et al. Fecal microbiota transplantation is safe and tolerable in patients with multiple sclerosis: A pilot randomized controlled trial. *Mult Scler J Exp Transl Clin* 2022; **8**(2): 20552173221086662.

15. Zhao HJ, Luo X, Shi YC, et al. The Efficacy of Fecal Microbiota Transplantation for Children With Tourette Syndrome: A Preliminary Study. *Front Psychiatry* 2020; **11**: 554441.

16. Mandrioli J, Amedei A, Cammarota G, et al. FETR-ALS Study Protocol: A Randomized Clinical Trial of Fecal Microbiota Transplantation in Amyotrophic Lateral Sclerosis. *Frontiers in neurology* 2019; **10**: 1021.