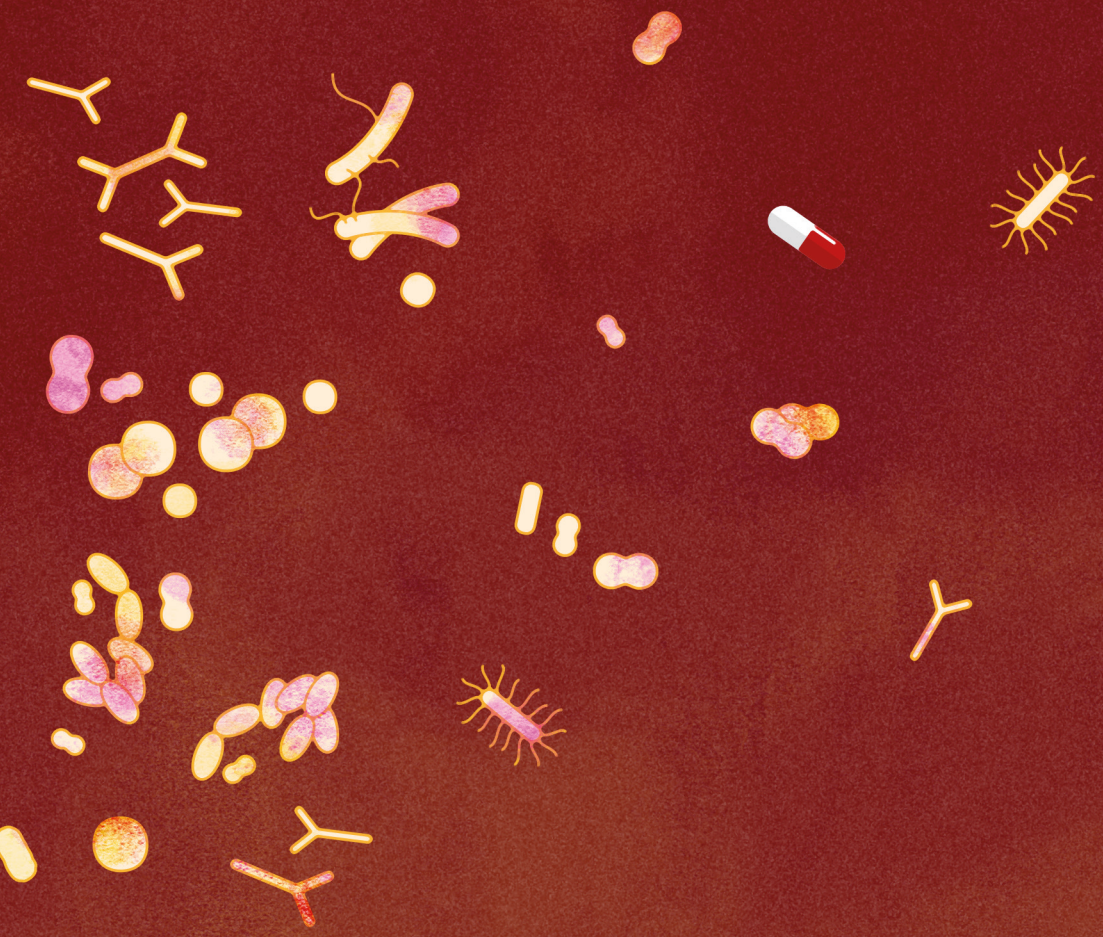
the epidemiology of infections with Clostridioides difficile and multidrug-resistant bacteria, and faecal microbiota transplantation as an intervention strategy

Vendrik, K.E.W.

Chapter 1

Introduction and thesis outline



Clostridioides difficile infections and the role of the gut microbiota

Epidemiological and clinical characteristics and treatment of *C. difficile* infections

Clostridioides difficile (C. difficile) is a spore-forming, anaerobic Gram-positive bacterium, which can colonise the intestines of humans and animals. Among healthy individuals in the community, 2-4% is asymptomatically colonised with *C. difficile* in their gut, as compared to 7-18% of patients admitted to the hospital.[1] Pathogenic *C. difficile* strains can produce toxin A (TcdA), toxin B (TcdB), and/or binary toxin (CDT). This may lead to damage of the intestinal wall and thereby gastrointestinal symptoms with severity depending on host susceptibility and the virulence of the infecting strain.[1] CDI often presents with mild diarrhoea. In some cases, more severe disease may develop, that may include bloody diarrhoea, pseudomembranous colitis, toxic megacolon, bowel perforation, sepsis and/or multiple organ dysfunction syndrome. Pseudomembranous colitis indicates a typical endoscopic picture with haemorrhage and deep ulcerations. Toxic megacolon involves severe dilatation of the colon and is characterised by systemic toxicity and high mortality.[1] In the Netherlands, 16% of hospitalised CDI patients suffer from severe CDI. Death within 30 days is reported for 9% of hospitalised CDI patients with 1% CDI-related mortality.[2]

Based on data of the National Expertise Centre for *C. difficile*, the CDI incidence rate in Dutch hospitals is relatively stable at around 3 CDI cases per 10,000 patient-days.[2] The mean hospital CDI incidence rate in Europe was 3.19 cases per 10,000 patient-days in 2016, ranging from 2.5 in Malta to 14.8 in Estonia.[3] However, the CDI testing frequency differed considerably between countries. The median number of CDI stool tests per 10,000 patient-days ranged between 19.6 in Croatia and 179.0 in the Netherlands. As expected, countries with the lowest CDI testing rates had the highest percentages of CDI positive stool tests.[3]

CDI is the most common cause of healthcare-associated gastro-intestinal infections.[4-6], but it is also frequently observed in the community.[7] In the last 10–20 years, the incidence of CDI in the community has increased, mainly in patient populations previously thought to be at lower risk, including younger patients and those without prior antibiotic exposure.[8] In the Netherlands, almost half of the hospitalised CDI cases have onset of symptoms in the community.[9] However, an important problem in the community is that CDI is frequently unrecognised since general practitioners tend to request less *C. difficile* diagnostics compared to physicians in the hospital.[10]

The most frequently found so called "hyper"virulent *C. difficile* ribotype is PCR ribotype (RT) 027. Compared to other ribotypes, it causes more severe disease, higher mortality and more recurrent CDI [11,12], which may reflect type-specific host susceptibility and/or an increased virulence of the strain.[13] In the Netherlands, *C. difficile* RT027 was detected for the first time[14] in 2005 and it rapidly spread with major hospital outbreaks.[11,15] These

events prompted the Centre for Infectious Disease Control of the National Institute for Public Health and the Environment (RIVM) to establish the national reference laboratory for *C. difficile* at the Leiden University Medical Centre. The *C. difficile* reference laboratory provided ad hoc PCR ribotyping services in case of a suspected outbreak or severely diseased patients and coordinated the *C. difficile* sentinel surveillance in the Netherlands with 21-24 participating Dutch hospitals. Since mid-2006, the occurrence of ribotype 027 in the Netherlands has decreased significantly.[9,16] At the start of 2022, the *C. difficile* reference laboratory was transformed into a *C. difficile* expertise centre with only five hospitals participating in the sentinel surveillance.

The most important risk factors for acquiring a *C. difficile* infection are the use of antibiotics, higher age, recent hospitalisation, female sex, proton pump inhibitor (PPI) use, having a feeding tube, being resident in a long-term care facility, steroid use and comorbidity.[17] All antibiotic classes may be associated with CDI,[18] but in the Netherlands the risk is greatest for (in order of risk) carbapenems, second and third-generation cephalosporins, metronidazole and broad-spectrum penicillin combinations with beta-lactamase inhibitors (after adjustment for confounders).[19] The use of more small-spectrum antibiotics considerably reduces the risk for CDI. Besides the antibiotic class, also the number of administered antibiotics, dosage and duration of therapy are associated with a higher risk for CDI.[20] Risk factors for severe CDI are higher age and the presence of multiple comorbidities.[21]

An accurate diagnosis of CDI is challenging.[22-24] The diagnosis is based on clinical suspicion in combination with a laboratory test that confirms the presence of CDI toxins or toxin genes in the stool of the patient. Several laboratory tests with different targets are available. However, none of these tests are both highly sensitive and specific in diagnosing CDI and have low hands-on time and low costs.[23] Detection of free toxins in stool by the use of a toxin Enzyme Immunoassay only could underestimate the CDI incidence due to the low sensitivity, but the use of a Nucleic Acid Amplification test only could overestimate the CDI incidence with frequent detection of asymptomatic carriers due to the low specificity.[24] The gold standard method is the cytotoxicity assay with good specificity and sensitivity, but this is not frequently performed due to the lack of standardisation and long turn-around time.[23] Therefore, a two- or three step algorithm is recommended by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), including a highly sensitive screening test and a highly specific confirmation test.[24] This is visualised in Figure 1.

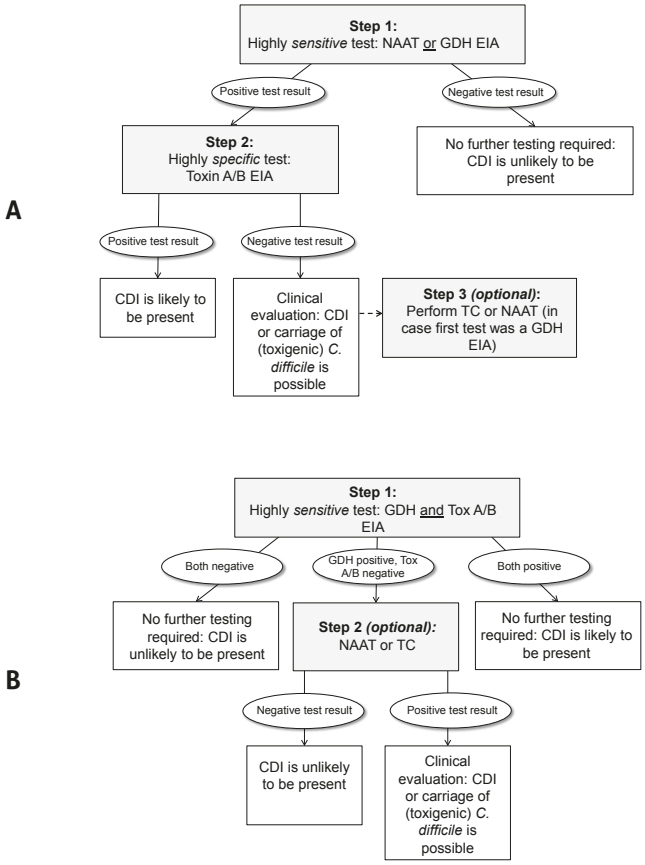


Figure 1. Algorithms for *C. difficile* testing, recommended by the European Society of Clinical Microbiology and Infectious Diseases.

Abbreviations: CDI: *Clostridioides difficile* infection, EIA: enzyme immunoassay, GDH: glutamate dehydrogenase, NAAT: nucleic acid amplification test, TC: toxigenic culture, Tox A/B: toxin A/B. Retrieved unchanged from Crobach MJT, *et al.*[24] © CC BY-NC-ND 4.0: https://creativecommons.org/licenses/by-nc-nd/4.0/.

C. difficile can easily spread via spores, which are resistant to heat and numerous other disinfectants.[17] In contrast to the vegetative cells of *C. difficile*, spores can survive for months in the environment. Transmission within the hospital setting is therefore common. Besides transmission of *C. difficile* via symptomatic CDI patients, transmission is also possible via asymptomatic carriers, although at a lower rate than symptomatic CDI patients.[25,26] Unfortunately, standard infection control precautions focus on symptomatic CDI patients only. The Dutch guidelines of the "Werkgroep Infectie preventie" (July 2011) and the guidelines (2018) from the ESCMID study group for *C. difficile* (ESGCD)

recommend application of contact precautions, personal protective equipment and daily and terminal environmental cleaning and disinfection of rooms and equipment that have been in contact with CDI patients[27]. For hand-hygiene, it is recommended to switch from alcohol-based hand rub to washing with soap and water in an outbreak setting, but not in an endemic situation.[28] Contact precautions are frequently ended 48 hours after the clearance of CDI symptoms, but CDI patients may still shed spores after this period.[29] Furthermore, high concentrations of chloride and a long exposure time are needed to eradicate spores, which is not enforced by many Dutch hospitals due to occupational health issues.[27] Antibiotic stewardship is also important in reducing CDI incidence.[30] The use of antibiotic agents should be restricted and, if used, it should be preferably small-spectrum. The duration of antibiotic therapy should be kept to a minimum.[28] Since 2015, Dutch hospitals have A-teams, responsible for implementation of antibiotic stewardship programs. Compared to other countries, the use of antibiotics is low in the Netherlands.[31] However, there is still progress to be made.

C. difficile infections are primarily treated by discontinuation of the inciting antibiotic and by prescribing antibiotics for which *C. difficile* is susceptible, such as metronidazole, vancomycin and fidaxomicin. Which antibiotic to choose depends on the severity of the disease, the number of previous CDI episodes, provided CDI antibiotics for previous episodes and the risk of recurrence. Since 2021, the use of metronidazole is no longer recommended by the ESCMID[32] and the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA),[33] due to its inferior cure rates and higher recurrence rates compared to vancomycin and fidaxomicin.[32] Fidaxomicin is the preferred treatment in case of an initial CDI episode or the first CDI recurrence. When this is not available or feasible and there is a high risk of recurrence, bezlotoxumab (humanised monoclonal antibodies against *C. difficile* toxin B) should be provided in addition to vancomycin. When fidaxomicin is prescribed for the initial CDI episode, bezlotoxumab could be added in case of a first recurrence. Bezlotoxumab or faecal microbiota transplantation (FMT) in addition to anti-CDI antibiotics are advised for treatment of patients with multiple recurrent CDI.[32]

Recurrent episodes of CDI are not uncommon. Approximately 20–35% of patients with a first CDI episode develop a recurrence and, of these, 40–60% have a second episode.[34] After the second recurrence, patients are prone to develop multiple recurrences.[35] Fortunately, (relatively) new treatment strategies such as fidaxomicin,[36] bezlotoxumab,[37] and FMT[38] have lowered the recurrence rates.

Metronidazole resistance in *C. difficile* strains is rare and resistance rates vary considerably between countries.[39] It appears to be at least partly plasmid-mediated.[40] Fortunately, vancomycin and fidaxomicin resistance is still rare.[41,42] Importantly, vancomycin resistance seems to increase *in vitro* with elevated minimal inhibitory concentrations (MIC) though confirmation is necessary and the clinical relevance is

unclear.[41] Furthermore, *C. difficile* strains are resistant to many other antibiotics and could act as a reservoir of antimicrobial resistance genes that could potentially be transferred to other pathogens.[39]

The role of the human gut microbiota in *C. difficile* infections

The gut microbiota is the assemblage of microorganisms present in the gut. Sometimes, articles refer to the gut microbiome, which includes the entire habitat, including the microorganisms (bacteria, archaea, eukaryotes, and viruses), their genomes, and the surrounding environmental conditions.[43]

CDI is characterised by a disturbed gut microbiota, which is frequently the result of previous use of antibiotics, an important risk factor for *C. difficile* infections.[17] However, several other factors, such as comorbidity, diet, travel and other medication (such as proton pump inhibitors, non-steroidal anti-inflammatory drugs, antidiabetics and chemotherapeutics),[44] are also known to affect the composition and stability of the gut microbiota.[45] A disturbed microbiota could result in decreased colonisation resistance, i.e. the role of the microbiota to provide a barrier against colonisation and expansion of potentially pathogenic microorganisms. Colonisation resistance depends on functional, synergistic relationships between gut microorganisms and host cells and their associated metabolites.[46] Antibiotic-induced disruption of the gut microbiota could persist for several months, indicating that patients remain susceptible to develop CDI for a long period after antibiotic treatment.[19,47] Patients with CDI or with asymptomatic *C. difficile* colonisation are shown to have a lower diversity and richness of their gut microbiota compared to healthy subjects.[48-51] The number of different species in their gut is reduced and their relative abundance is less evenly spread (within-sample diversity; alpha diversity). Furthermore, their gut microbiota composition is different from healthy controls (between sample diversity; beta diversity)[52]. Patients with CDI have a different gut microbiota composition compared with asymptomatic *C. difficile* carriers with a significantly higher relative abundance of *Escherichia/shigella*,[50] *Clostridioides*, and *Veillonella*,[51] and lower abundance of *Bifidobacterium*[50] and genera belonging to the *Ruminococcaceae* family and Actinobacteria phylum[51] in CDI patients, although this differs between studies. Many of the genera that were lower abundant are known short-chain fatty acids (SCFA)-producers and carbohydrate degraders.[51] The disturbances of the gut microbiota are larger for patients with recurrent CDI, compared to patients with an initial CDI episode.[53]

Mechanisms by which the gut microbiota can mediate colonisation resistance against *Clostridioides difficile* are shown in Figure 2. Potential mechanisms by which a disruption of the microbiota may contribute to the development of CDI are alterations in bile acid or amino acid metabolism, production of bacteriocins or short chain fatty acids (mainly butyrate), metal availability, nutrient or niche competition, bacteriophage exposure, carbohydrate digestion and interactions with the host immune system.[54-56]

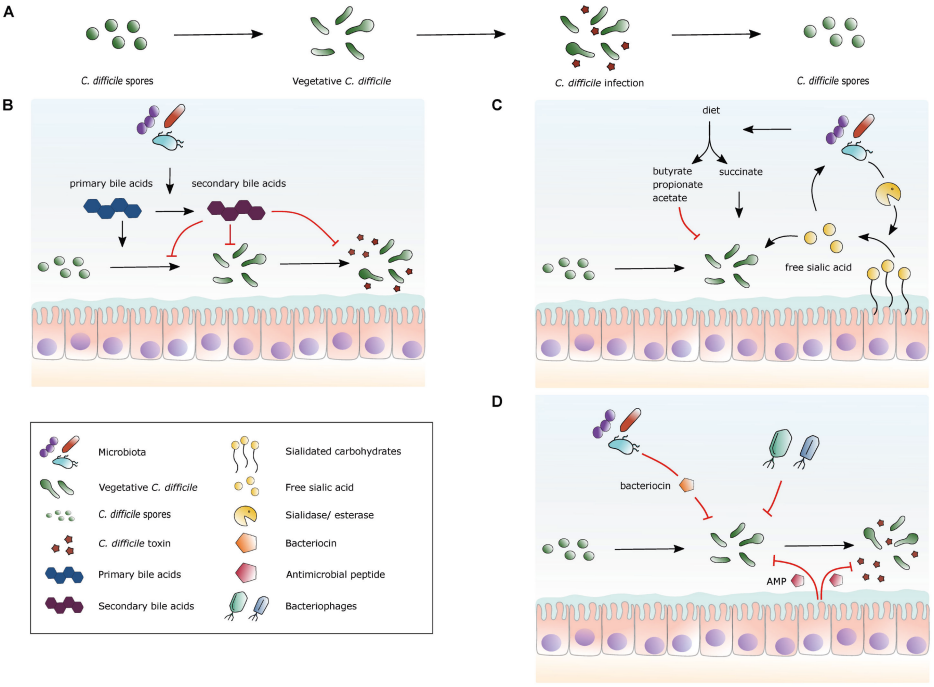


Figure 2. Several important mechanisms by which the gut microbiota can mediate colonisation resistance against *Clostridioides difficile*.

Part A describes the lifecycle of *C. difficile* from spores to vegetative cells to toxin-producing cells and back to spores. Part B shows the effects of the bile acid metabolism on *C. difficile*. Primary bile acids stimulate *C. difficile* spore germination into vegetative cells. Members of the gut microbiota in the colon can metabolise these primary bile acids into secondary bile acids. Secondary bile acids inhibit spore germination and toxin production. Part C shows the effects of certain nutrients on *C. difficile*. Short-chain fatty acids (SCFAs) are produced by the gut microbiota through microbial fermentation of dietary fibre and starches. Most SCFAs inhibit growth of *C. difficile*, whereas succinate can stimulate *C. difficile* expansion. Sialic acid is released by cleavage of sialidated carbohydrates by certain members of the gut microbiota. Sialic acid stimulates *C. difficile* expansion. Part D shows the effects of antimicrobial factors. Some members of the gut microbiota produce bacteriocins, that can be bacteriostatic or bactericidal for *C. difficile*. Furthermore, *C. difficile* can be used as a host for some bacteriophages in the gut microbiota. In addition, host cells can produce antimicrobial peptides (AMP) that inhibit *C. difficile* growth and toxin activity. [56]

Abbreviations: AMP: antimicrobial peptides.

The exact members of the microbiota involved in protection against or development of CDI have not been elucidated yet. However, several micro-organisms and metabolites have been suggested to play a role. Studies suggest that CDI appears to be associated with a decrease in members of the phyla Bacteroidetes and Firmicutes and an increase in Proteobacteria and Bacilli.[53,57] Proteobacteria contain numerous pathogenic pro-inflammatory bacteria, such as the genera *Escherichia*, *Klebsiella* and *Enterobacter.*[58] Bacteroidetes are involved in carbohydrate digestion and they produce short-chain fatty acids, mainly propionate and acetate.[59] They produce substrates essential for homeostasis of colonocytes and thereby colonic health.[60] The phylum Firmicutes includes several butyrate-producers and bile acid metabolising bacteria. CDI patients typically have a reduced number of butyrate-producing commensal bacteria in their gut.[48] Butyrate is an SCFA, which is produced by the gut microbiota through microbial fermentation of dietary fibre and starches in the lower intestinal tract. Butyrate has anti-inflammatory and pH-lowering properties (while *C. difficile* favours an alkaline pH[61]) and enhances intestinal barrier function and mucosal immunity.[62] Butyrate-producing bacteria, such as *Lachnospiraceae* and *Ruminococcaceae* of the phylum Firmicutes, are thought to provide colonisation resistance against CDI.[48,52] The germination of *C. difficile* spores can be influenced by bile salts. Primary bile acids are produced in the liver and are released into and reabsorbed from the small intestines. Primary bile acids stimulate *C. difficile* spore germination. A small proportion of primary bile acids is not reabsorbed in the small intestines and is passed into the colon. Members of the gut microbiota in the colon can metabolise these primary bile acids into secondary bile acids. Secondary bile acids inhibit the growth of *C. difficile.*[55] The gut microbiota members that can metabolise primary bile acids into secondary bile acids are of the *Lachnospiraceae, Ruminococcaceae* and *Blautia* families of the phylum Firmicutes.[63,64] A decrease in these taxa through disruption of the microbiota will result in reduced secondary bile acid levels and increased primary bile acid levels with a favourable environment for *C. difficile.* CDI patients indeed have higher levels of primary bile acids and lower levels of secondary bile acids in their faeces, compared to controls, mainly in recurrent cases.[65] In an *in vitro* study and a mouse study, *Clostridium scindens*, a bile acid 7α-dehydroxylating commensal intestinal bacterium, appeared to play a role in the resistance against CDI by converting primary bile acids to secondary bile acids and by the production of antimicrobials.[66,67] However *C. scindens* has also been found in stool samples of CDI patients,[68] although this study is under investigation for scientific misconduct.[69] The amount of germination in response to bile acids depends on whether there is a functional CspC germinant receptor that recognises primary bile acids.[70]

FMT as treatment strategy to prevent recurrent episodes of *C. difficile* infections

The decreased colonisation resistance due to the disturbed microbiota in CDI patients can be counteracted by using microbiota interventions, such as FMT,[38] probiotics[71] and live biotherapeutics.[72-76] Live biotherapeutics are a recent development, aiming to replace FMT as microbiota intervention. They contain established consortia of live microorganisms that are proven to have a beneficial effect on human diseases. RBX2660[72] is the first live biotherapeutic that has recently been approved by the FDA, though it is not based on cultured microorganisms but it contains merely a well characterised donor faecal suspension. Furthermore, studies on SER-109[73] are at an advanced stage and have also shown promising results. As mentioned, bezlotoxumab or FMT in addition to anti-CDI antibiotics are currently advised as treatment in patients with multiple recurrent CDI.[32] At the moment, CDI is the only registered treatment indication for FMT. Besides recurrent CDI, FMT can also be provided for patients with severe CDI,[77,78] although more evidence is needed to ascertain the chance of improvement and the safety in this fragile population.[79] The cure rate of FMT for patients with multiple recurrent CDI is 83-92%.[80-82] For some patients, repeated FMTs are required.

FMT restores the reduced gut microbiota diversity and the disturbed composition and metabolic capacity of the microbiota in CDI patients by administration of a healthy gut microbiota of a faeces donor.[83-85] Thereby, colonisation resistance against *C. difficile* will be improved. However, some evidence suggests it is not the donor microbiota that provides the cure, since sterile faecal filtrates[86] and autologous FMT[87] appear to be beneficial as well.

Broad-spectrum antibiotics and other microbiota-disturbing factors lead to disruption of the gut microbiota. After exposure to *C. difficile*, this may lead to a vicious cycle with (recurrent) *C. difficile* infection for which antibiotic treatment is provided and persistent dysbiosis, which can be restored by FMT.[88] After FMT, the microbiota of the patient is at least partly replaced by the healthy donor microbiota and a new balance will be achieved with a patient microbiota that is more similar to the microbiota of the donor.

The mechanisms underlying the success of FMT in recurrent CDI are only partly understood. The microbiota composition of successfully treated patients changes after FMT from a low-diversity microbiota which is dominated by Proteobacteria and Bacilli, to a more diverse microbiota resembling that of healthy donors, which is dominated by Bacteroidetes and *Clostridium* groups, including butyrate-producing bacteria.[57] Furthermore, FMT leads to restoration of the bile acid balance by an increase in secondary bile acid levels and decrease in primary bile acid levels.[84] Indeed, bile acid concentrations from patients' faeces prior to FMT induce *C. difficile* germination, while bile acid concentrations from patient's faeces after FMT do not induce germination and inhibit vegetative growth of *C. difficile* strains.[89] Fungi may also play a role in the success

of FMT,[90,91] although evidence is unclear for fungi other than *Candida albicans*. CDI patients frequently have a high abundance of *Candida albicans* in their faeces and a decreased fungal diversity, richness and evenness, which are improved after a successful FMT to a state comparable to that of the donor. In faeces of non-responders, *C. albicans* remains dominant, and faeces of donors enriched with *C. albicans* are associated with reduced FMT efficacy.[91] There may also be a role for other non-bacterial, non-fungal components of faecal suspensions, such as viruses and bacteriophages,[86] in the success rate of FMT in CDI.

Faeces donors, who provide faeces for FMT, are usually rigorously screened with risk assessments and repeated blood and faecal examination.[92] Their donated faeces is processed into a faecal suspension, which is usually stored in a -80 degrees Celsius freezer until use. The faecal suspensions are administrated via upper or lower gastrointestinal (GI) routes. Potential upper GI routes include oral capsules, nasogastric tube, nasoduodenal tube, nasojejunal tube or direct gastric/duodenal/jejunal administration via gastroscope; lower GI routes include enema or direct infusion into the rectum or colon via colonoscope. The lower GI routes appear slightly more effective,[81,82] but are associated with slightly more serious adverse events compared to upper GI routes.[93] FMT treatment frequently includes pre-treatment with anti-CDI antibiotics and bowel lavage to improve engraftment of the donor gut microbiota. When a CDI recurrence develops post-FMT, this can be treated with anti-CDI antibiotics (preferably a small spectrum agent such as fidaxomicin) alone due to the improved state of the gut microbiota post-FMT. Risk factors for a CDI recurrence after FMT include the use of non-CDI antibiotics after FMT and moderately or severely immunocompromised state.[94]

FMT is considered a rather safe treatment for patients with CDI (although underreporting of serious adverse events is likely[95]).[82] FMT-related serious adverse events (SAEs) in these patients, such as aspiration pneumonia, infections, GI haemorrhage or death, have been described, but occur in no more than 5% of patients.[93,94,96,97] A study performed by the Netherlands Donor Feces Bank on FMT-treated patients with recurrent CDI revealed that approximately 21-33% of patients report mild GI adverse events (AEs), such as abdominal pain and diarrhoea, in the three weeks after FMT and at long-term follow-up.[94] A recent meta-analysis found an AE rate of 39%.[97] These AEs are mostly self-limiting and may also (partly) be a result of the CDI itself or post-infectious irritable bowel syndrome.[98] Long-term SAEs are largely unknown, although one recent study suggests that FMT does not cause long-term SAEs.[99]

Multidrug-resistant bacteria and the role of the gut microbiota

Epidemiological and molecular characteristics of multidrug-resistant bacteria

Antimicrobial resistance (AMR) is one of the biggest threats to public health. It can occur in bacteria, fungi, parasites, and viruses. This thesis focusses on antimicrobial resistance in bacteria. Bacteria can be intrinsically resistant or they can acquire AMR. AMR can be acquired by mutations in chromosomal genes or by acquisition of exogenous resistance genes that are carried on mobile genetic elements.[100] Mobile genetic elements are DNA segments that encode enzymes mediating the movement of other DNA pieces.[101] They can be divided into two groups: 1) those enabling intracellular mobility with movement of DNA segments within the same bacterial genome including insertion sequences, transposons, integrons and prophages, and 2) those enabling intercellular mobility with transfer of genes between different bacteria including plasmids and integrative conjugative elements. A plasmid is extra-chromosomal circular DNA that replicates independently of the host genome and that can also spread horizontally between bacteria, including bacteria from different species.[102] This means that AMR genes can be transferred from commensals in the gut, to pathogenic bacteria that can be life-threatening to humans after infection. Transfer of plasmids with AMR genes are of the most concern.

The use of antibiotics and transmission of antimicrobial resistant micro-organisms (or plasmids) between humans, animals and the environment are the most important drivers of AMR. AMR is therefore regarded a One Health issue. The spread can be restricted by appropriate infection prevention control measures and prudent antimicrobial prescribing, comprising limited use and short duration of antibiotics that are as small spectrum as possible.[100] It is important that prudent and limited antimicrobial prescribing is also implemented in animals, such as pets and livestock. Antibiotics are used in livestock to treat infections, but also as feed additives and growth promotors. Furthermore, multiple environmental reservoirs play a role in AMR dissemination, including soil, water, waste of hospital, industries and farms, and various polluted ecological niches.[103] A coordinated One Health approach is essential in the combat against AMR.

The increase in multidrug-resistant bacteria (MDRO), bacteria that are resistant to multiple clinically relevant antibiotics and that can spread worldwide, is worrisome. Antibiotic resistant bacteria that belong to the so-called ESKAPE group (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanni, Pseudomonas aeruginosa* and *Enterobacter* species) are the most threatening and are associated with hospital infections with high mortality rates.[104] In the Netherlands, there are relatively few problems with MDRO so far.[100 105]

Infections with MDRO are frequently preceded by intestinal colonisation with this MDRO.[46] However, individuals can also remain asymptomatically colonised.

Unfortunately, definitions of MDRO differ between countries and regions. The currently published guideline of the Dutch Working Group on Infection Prevention[106] (a new guideline is in progress but is not published yet) defines MDRO as:

- Extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales, Enterobacterales that are resistant to both a fluoroquinolone and an aminoglycoside or that produce carbapenemases.
- *Acinetobacter* spp that are resistant to both a fluoroquinolone and an aminoglycoside or that produce carbapenemases.
- *Pseudomonas aeruginosa* that is carbapenemase-producing and resistant to at least two of the following antibiotic classes or agents (or no carbapenemase production and a combination of three classes or agents): fluoroquinolones, aminoglycosides, ceftazidime or piperacillin, and carbapenems.
- Co-trimoxazole-resistant *Stenotrophomonas maltophilia* (outside the scope of this thesis).
- Penicillin- and vancomycin-resistant *Enterococcus faecium* (VRE).
- Penicillin- or vancomycin-resistant *Streptococcus pneumoniae* (outside the scope of this thesis).
- Meticillin-resistant *Staphylococcus aureus* (MRSA).[106]

Colistin-resistant Enterobacterales are also of serious concern since colistin is a last-resort treatment option against ESBL-producing and carbapenem-resistant Enterobacterales.

Multidrug-resistant Gram-negatives

Multidrug-resistant Gram-negative bacteria are rapidly emerging worldwide. Beta-lactam antibiotics, such as penicillins, cephalosporins and carbapenems, are among the most frequently prescribed antibiotics worldwide. These antibiotics bind to and inhibit bacterial enzymes involved in cell wand synthesis.[107] However, some bacteria contain bacterial enzymes, called beta-lactamases, that inactivate beta-lactam antibiotics by hydrolysis. This is the most important contributing factor to beta-lactam resistance.[102] ESBLs are a group of beta-lactamases that cause resistance to oxyimino-cephalosporins (e.g. ceftazidime or cefuroxime) and monobactams (e.g. aztreonam), but not to cephamycins (e.g. cefoxitin) or carbapenems (e.g. meropenem or imipenem) and they are inhibited by beta-lactamase inhibitors (e.g. clavulanic acid).[108] There is an increase in penicillin and cephalosporin resistance due to the global spread of ESBL-producing bacteria. The most important ESBL genes include several types of $bla_{CTX\text{-}M}$, bla_{TEM} and bla_{SHV}. These genes can mobilise intracellularly via insertion elements, transposons and integrons, but they can also spread between different bacteria by plasmids.[102] Figure 3 and 4 show resistance to third generation cephalosporins (e.g. ceftazidime and ceftriaxone) of invasive *E. coli*

and *K. pneumoniae* isolates in Europe in 2020. The population-weighted mean resistance percentage for the European Union/European Economic Area (EU/EEA) was 14.9% in 2020 and 13.8% in 2021 for invasive *E. coli* isolates, with resistance percentages of 50.0% or above in 12.5% of countries in 2020. For invasive *K. pneumoniae* isolates, the mean resistance percentage was 33.9% in 2020 and 34.3% in 2021, with 43.9% of countries reporting resistance percentages of ≥50% in 2020. Ceftazidime resistance was observed in 15.5% of European invasive *Pseudomonas aeruginosa* isolates in 2020 and 15.8% in 2021.[100,109]

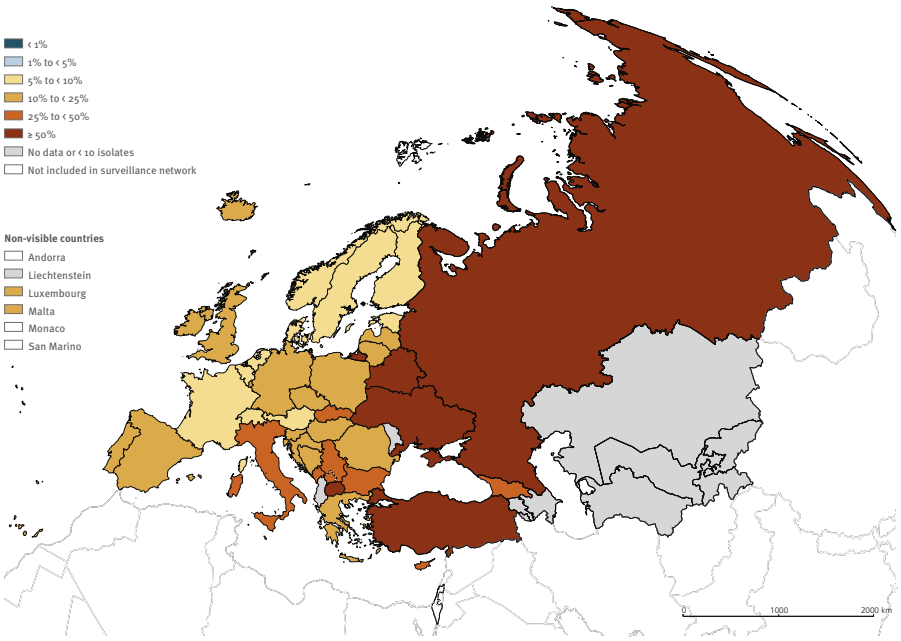


Figure 3. Percentage of invasive *E. coli* isolates resistant to third-generation cephalosporins in Europe in 2020.

Note: data for Serbia and Kosovo were combined for this map. Data for the United Kingdom for 2020 do not include Scotland and Wales. Data sources: 2020 data from the Central Asian and European Surveillance of Antimicrobial Resistance (©WHO 2021. All rights reserved.) and 2020 data from the European Antimicrobial Resistance Surveillance Network (©ECDC 2021). Map production: ©WHO.

Retrieved unchanged from World Health Organization Regional Office for Europe/European Centre of Disease Prevention and control.[100] © CC BY 3.0 IGO: https://creativecommons.org/licenses/by/3.0/igo/.

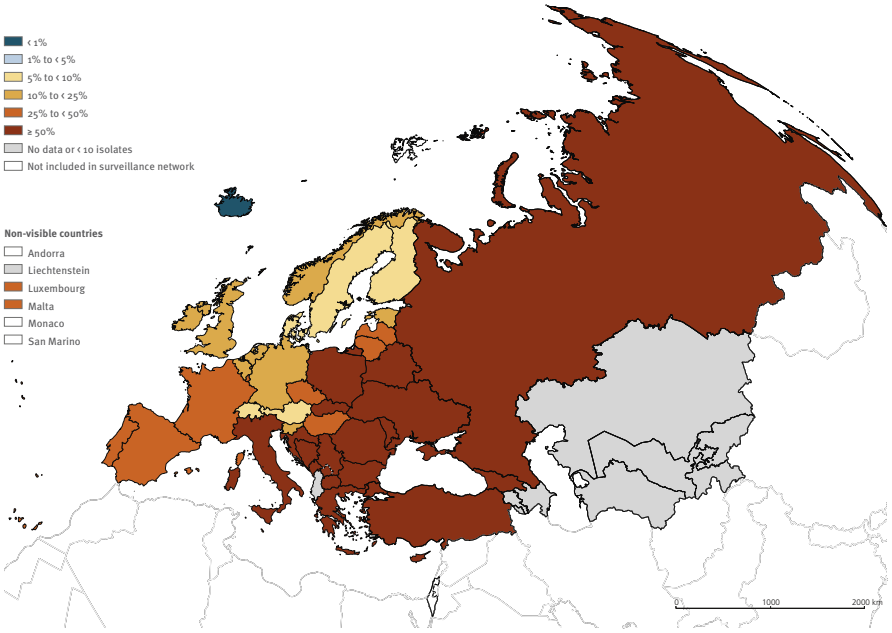


Figure 4. Percentage of invasive *K. pneumoniae* isolates resistant to third-generation cephalosporins in Europe in 2020.

Note: data for Serbia and Kosovo were combined for this map. Data for the United Kingdom for 2020 do not include Scotland and Wales. Data sources: 2020 data from the Central Asian and European Surveillance of Antimicrobial Resistance (©WHO 2021. All rights reserved.) and 2020 data from the European Antimicrobial Resistance Surveillance Network (©ECDC 2021). Map production: ©WHO. Retrieved unchanged from World Health Organization Regional Office for Europe/European Centre of Disease Prevention and control.[100] © CC BY 3.0 IGO: https://creativecommons.org/licenses/by/3.0/igo/.

Another type of beta-lactamase is the ampC type (AmpC-BL), that causes decreased susceptibility to oxyimino-cephalosporins and methoxy-cephalosporins, but with good susceptibility against fourth-generation cephalosporins. The phenotypic resistance is very similar to ESBL-producers, except that AmpC-BL is not inhibited by clavulanic acid. Several bacteria contain these genes on their chromosomes, but some species, such as *E. coli* and *K. pneumoniae*, can present these genes on plasmids.[110]

Carbapenems are beta-lactam antibiotics, such as meropenem and imipenem, that are effective against ESBL-producing bacteria. Unfortunately, carbapenem-resistance is also spreading worldwide. Carbapenem-resistance is for a large part caused by carbapenemase-encoding genes, such as bla_{NDM}, bla_{KPC}, bla_{VIM}, bla_{IMP}, and bla_{OXA-48}. Carbapenemases are beta-lactamase enzymes that hydrolyse carbapenems. These genes are frequently located

on plasmids or associated with other mobile genetic elements, like transposons and integrons. A cause of concern is the occurrence of a combination of carbapenemase genes with multidrug resistance genes on the same mobile genetic element.[111] Other causes of carbapenem resistance include the combined effect of other beta-lactamases and/or decreased bacterial cell membrane permeability due to alterations or mutations in porins, increased transport out of the cell by efflux-pumps or modification of targets of antibiotics through genetic mutations or post-translational modification.[111,112]

The population-weighted EU/EEA mean resistance percentage for carbapenem among invasive *Klebsiella pneumoniae* isolates in Europe was 10.0% in 2020 and 11.7% in 2021, with 15% of European countries reporting resistance percentages of 50.0% or higher in 2020. This is shown in Figure 5.

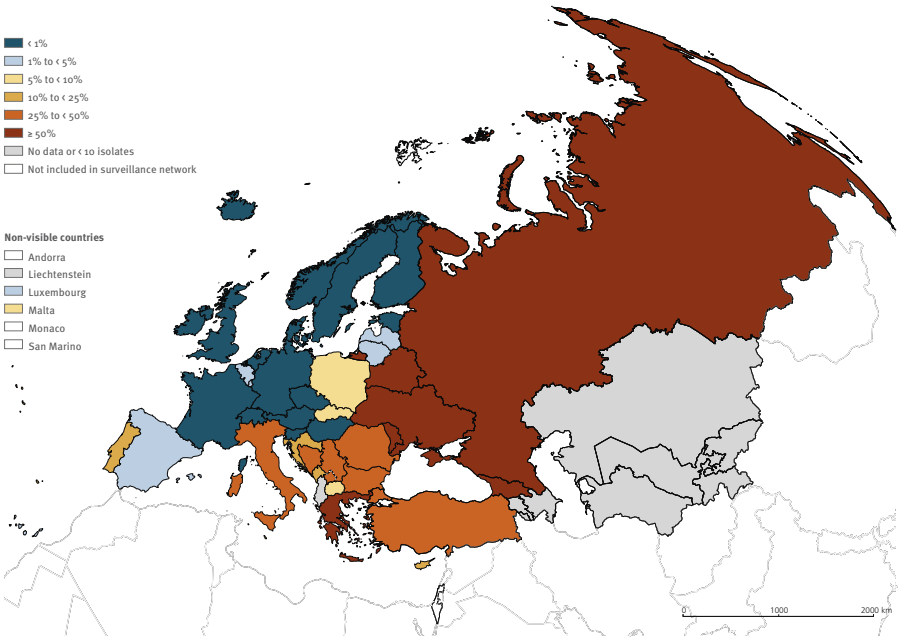


Figure 5. Percentage of carbapenem-resistant invasive *K. pneumoniae* isolates in Europe in 2020.

Note: data for Serbia and Kosovo were combined for this map. Data for the United Kingdom for 2020 do not include Scotland and Wales. Data sources: 2020 data from the Central Asian and European Surveillance of Antimicrobial Resistance (©WHO 2021. All rights reserved.) and 2020 data from the European Antimicrobial Resistance Surveillance Network (©ECDC 2021). Map production: ©WHO.

Retrieved unchanged from World Health Organization Regional Office for Europe/European Centre of Disease Prevention and control.[100] © CC BY 3.0 IGO: https://creativecommons.org/licenses/by/3.0/igo/.

It was only observed in 0.2% of European invasive *Escherichia coli* isolates in 2020 and 2021. Significant increasing trends are observed for both *K. pneumoniae* and *E. coli* in Europe. Carbapenem resistance is also frequently observed in invasive isolates of *P. aeruginosa* and *Acinetobacter species*, even at a higher percentage than in invasive *K. pneumoniae* isolates.[100,109]

Polymyxins are one of the last resort treatment options for infections with carbapenem-resistant multidrug-resistant (MDR) Gram-negative bacteria. Polymyxins are cationic polypeptide antibiotics that bind to lipopolysaccharides (LPS) and phospholipids in the outer cell membrane of Gram-negative bacteria. They displace divalent cations by competition from the phosphate groups of membrane lipids, leading to disruption of the outer cell membrane and ultimately cell death.[113,114 115] Furthermore, polymyxins can bind to and neutralise LPS, reducing the pathophysiologic effects of this endotoxin in the circulation.[116,117]

There are two polymyxins available for the treatment of patients: polymyxin E (colistin) and polymyxin B. The antibacterial potencies of polymyxin B and colistin are identical. In the Netherlands, colistin is used orally for selective digestive tract (SDD) or oropharyngeal (SOD) decontamination. Furthermore, colistin in nebulised form is used for treatment of *Pseudomonas aeruginosa* colonisation/infection in patients with pulmonary diseases (mainly cystic fibrosis), topically for otitis externa or ophthalmic infections and parenterally for treatment of severe infections with MDRO or in cystic fibrosis patients. However, colistin is potentially neuro- and nephrotoxic when administered parenterally.[118] Colistin also used in livestock to treat infections caused by Enterobacterales, and in the past (and still in some non-European countries[119]) also as growth promotor.[120]

Unfortunately, colistin resistance has also been developed.[121-123] Some Gram-negative species are intrinsically resistant to colistin, such as *Neisseria meningitides, Burkholderia* species, and *Proteus mirabilis*, [124] but colistin resistance can also be acquired. Colistin-resistance can be caused by several mechanisms, such as modification of lipid A in LPS due to chromosomal mutations, expression of a plasmid-mediated mobilised colistin resistance (*mcr*) gene, loss of LPS from the cell membrane, hyperproduction of capsule polysaccharides or the activity of efflux pumps.[124]

Several chromosomal mutations in bacteria can lead to colistin resistance. For *K. pneumoniae*, mutations in the chromosomally located *pmrAB, phoPQ, mgrB* and *crrB* genes have been intensively studied. Mutations in these genes lead to modification of lipid A in LPS. This modification leads to decreased negative charge of the bacterial membrane impairing the interaction between colistin and LPS.[124] In *E. coli*, evidence on the role of chromosomal mutations in colistin resistance is scarce.[125] Colistin resistance in *E. coli* strains has been linked to *phoPQ* and *pmrAB* genes, but experimental confirmation is still mostly lacking.[125]

The risk for spread of colistin resistance is further increased by transferable plasmid-mediated *mcr* genes that can transmit colistin resistance more easily between bacteria, including bacteria from different species.[126] The first *mcr* gene, *mcr-1*, was discovered in China in 2015.[127] *Mcr* genes encode phosphoethanolamine transferases, which catalyse the addition of the cationic phosphoethanolamine group to the lipid A proportion of LPS (Figure 6).[124]

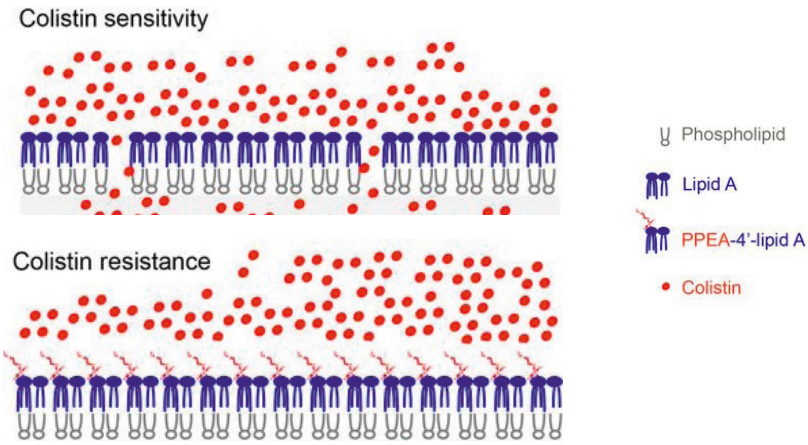


Figure 6. Colistin resistance by *mcr* genes.

Modification of the lipid A proportion of lipopolysaccharides of the outer membrane of Gram-negative bacteria by addition of phosphoethanolamine (PPEA) leads to a more cationic state with decreased affinity for cationic colistin.
Adapted (only part of the figure was used) from 2018 Xu Y, *et al.*[128] © CC BY 4.0: https://creativecommons.org/licenses/by/4.0/.

Since 2016,[127] *mcr* genes 1 to 10 have been discovered. However, it is debatable whether *mcr-9* leads to colistin resistance. In a study[129] examining *Enterobacter* isolates, none of the *mcr-9* carrying isolates had phosphoetanolamine modification. In a retrospective analysis of *E. coli* isolates from chicken origin in 2016, Shen *et al.*[130] found that *mcr-1* genes were already observed in the 1980s. Notably, *E. coli* is the most abundant *mcr*-containing species.[131,132] A study that examined 457 *mcr-1*-positive Enterobacterales isolates from 31 different countries, reported 411 *E. coli* isolates (89.9%).[131] Plasmid-transferable *mcr* genes are rare in other species than *E. coli* and *K. pneumoniae.*

Assessing the prevalence of colistin resistance via international or national surveillance is difficult, since colistin susceptibility testing is frequently not part of routine susceptibility testing and the performance of tests varies considerably.

K. pneumoniae is the species most commonly involved in the development of colistin resistance.[133] Among 646 carbapenem-resistant *K. pneumoniae* found in Europe in 2013-2014, 28% were also colistin-resistant.[134] According to the European Centre for Disease Prevention and Control (ECDC), 8.5% of tested *K. pneumonia* isolates in Europe in 2016 was colistin-resistant, of which 88.5% was from Greece and Italy. Colistin resistance was reported only sporadically in *E. coli* and *P. aeruginosa* and was found in 4.0% of tested *Acinetobacter spp.*, of which 70.7% derived from Greece and Italy. However, due to the low number of isolates and the frequent unreliable results of colistin susceptibility testing methods, these percentages should be interpreted with caution.[135] In order to obtain a better insight into the prevalence of colistin-resistance in Europe, ECDC initiated a survey on carbapenem- and/or colistin-resistant Enterobacterales (CCRE-survey) in 2019 in EU Member states.[136]

Multidrug-resistant Gram-positives

MDR Gram-positives of concern in the Netherlands are meticillin-resistant *Staphylococcus aureus* (MRSA) and penicillin- and vancomycin-resistant *Enterococcus faecium* (VRE).

Humans are frequently asymptomatically colonised with *S. aureus* on their skin and mucous membranes, including the nose and gut.[137] About 20% of humans are persistent nasal carriers and 30% are intermittent carriers.[138] *S. aureus* infections are frequently caused by *S. aureus* strains that these individuals have been carrying on the skin or mucous membranes.[139] *S. aureus* can cause infections of the bloodstream, skin and soft tissues and lower respiratory tract. It is an important nosocomial pathogen and can cause infections associated with medical instruments, such as central-line-associated bloodstream infections, or implantable materials, such as pacemakers and prostheses, as well as serious deep-seated infections such as osteomyelitis and endocarditis.[140]

A problem with *S. aureus* is the rapid development of resistance to multiple antibiotic classes. Within two years after the introduction of penicillin, the first penicillin-resistant *S. aureus* strain was detected.[141] Later, the semi-synthetic beta-lactamase-resistant antibiotic meticillin was introduced, but an MRSA was soon thereafter identified.[142] MRSA is resistant to all available penicillins and almost all of the other beta-lactam antibiotics. The resistance is caused by an acquired *mec* gene (*mecA*, *mecB, mecC* and *mecD*) located on the chromosome or on plasmids. The *mec* gene encodes an altered penicillin-binding protein with decreased affinity for most semisynthetic penicillins.[143,144] The *mec* gene is localised on a mobile genetic element termed "staphylococcal cassette chromosome *mec*" (SCC*mec*).[145]

S. aureus strains can enhance their virulence by various virulence factors, including toxins (such as exfoliative toxins, toxic shock syndrome toxin 1, enterotoxins and leukocidins), immune-evasive surface factors (such as capsule and protein A) and enzymes promoting tissue invasion (such as hyaluronidase).[146] One of the most studied

virulence factors is the cytotoxin Panton-Valentine Leukocidin (PVL). PVL encoding genes are possibly associated with development of skin infections, soft tissue infections and necrotising pneumonia, although evidence is contradicting.[146]

Infections with MRSA are associated with increased mortality rates, compared to meticillin-susceptible strains.[147,148] This can be caused by increased virulence of MRSA, but it is more likely that this is influenced by confounders/mediators such as increased age, more severe illness or comorbidity or delayed and less adequate treatment in MRSA patients.[148,149] In the past, MRSA was only associated with infections in health care facilities, but community-associated MRSA (CA-MRSA) infections have been emerging as well. At the time of the emergence of CA-MRSA strains, it was mostly limited to mild skin and soft tissue infections, but CA-MRSA strains have also been detected in health care facilities causing nosocomial infections. CA-MRSA strains have increased virulence and fitness,[150] are resistant to fewer non-beta-lactam antibiotics, carry a smaller version of SCC*mec*, and more frequently carry PVL encoding genes, compared to hospital-associated MRSA strains.[140]

The population-weighted EU/EEA mean resistance percentage for meticillin resistance among invasive *S. aureus* isolates in Europe was 16.7% in 2020 and 15.8% in 2021. A decrease in MRSA percentages was observed in the period of 2016-2021 from 19.3% to 15.8%.[100,109] Twenty-five percent of European countries had MRSA percentages of 25% or higher in 2020 (Figure 7).[100]

Another important MDR Gram-positive bacterium is VRE. Most enterococci that cause infection originate from the gut microbiota. Intestinal colonisation with VRE mostly precedes infection.[151] VRE infections are predominantly observed as urinary tract infections (UTIs), wound infections, intra-abdominal infections, bloodstream infections, endocarditis or catheter-related infections.[152]

Vancomycin resistance in enterococci is associated with *van* gene clusters (*vanA*, *vanB*, and so on). Expression of these gene clusters causes alteration of the vancomycin target on the cell wall, preventing vancomycin from binding to the cell wall.[153] The most important gene is *vanA*, whereas *vanB* clones are emerging.[154] These gene clusters can be located on plasmids or the chromosome.[155]

The population-weighted EU/EEA mean resistance percentage for invasive VRE isolates in Europe was 16.8% in 2020 and 17.2% in 2021. This has significantly increased compared to 2016, when it was 11.6%.[100,109] Importantly, it is estimated that the median number of infections and deaths attributable to vancomycin-resistant enterococci had almost doubled between 2007 and 2015,[156] which has probably increased more from 2016 onwards. In 11% of European countries resistance percentages of 50% or higher were observed in 2020.[100]

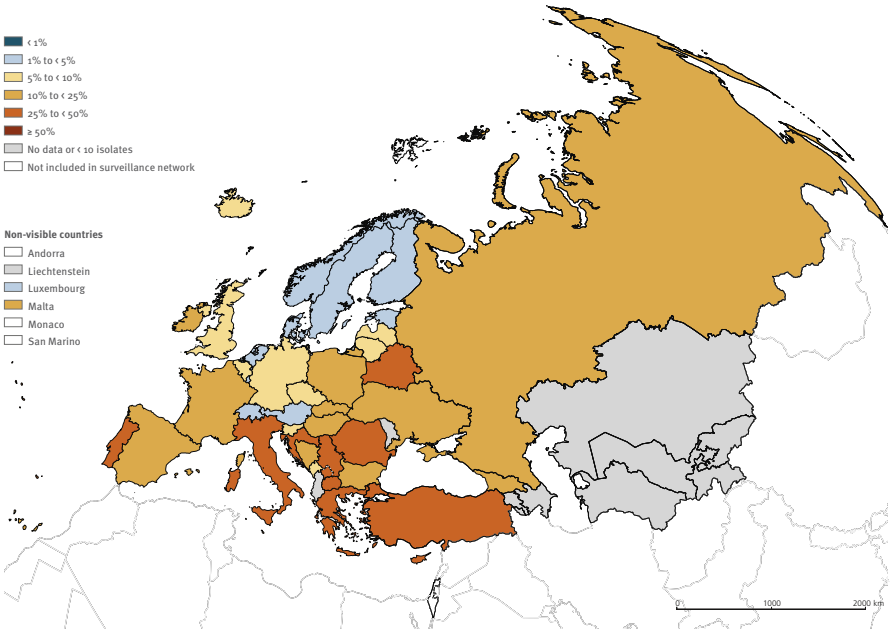


Figure 7. Percentage of invasive meticillin-resistant *S. aureus* isolates in Europe in 2020.

Note: data for Serbia and Kosovo were combined for this map. Data for the United Kingdom for 2020 do not include Scotland and Wales. Data sources: 2020 data from the Central Asian and European Surveillance of Antimicrobial Resistance (CAESAR, ©WHO 2021. All rights reserved.) and 2020 data from the European Antimicrobial Resistance Surveillance Network (EARS–Net, ©ECDC 2021). Map production: ©WHO.
Retrieved unchanged from World Health Organization Regional Office for Europe/European Centre of Disease Prevention and control.[100] © CC BY 3.0 IGO: https://creativecommons.org/licenses/by/3.0/igo/.

MDRO in the Netherlands

Compared to other countries, there are relatively few problems with MDRO so far in the Netherlands.[100 105] This can be explained by the relatively limited use of antibiotics in the Netherlands.[31] As depicted in Figures 3,4,5 and 7, the percentages of invasive *K. pneumoniae* and *E. coli* isolates that are resistant to third-generation cephalosporins or carbapenems, and the percentage of invasive MRSA isolates in the Netherlands are among the lowest percentages in Europe.[100]

In 2021, Nethmap reported a percentage of ESBL-producing isolates among diagnostic *E. coli* isolates of inpatient departments, intensive care units, outpatient departments and general practitioners of 5%, 9%, 4% and 3%, respectively. This was 8%, 15%, 6% and 3% for *K. pneumoniae*. These percentages have decreased compared to 2019 for most

patient groups, except for *K. pneumoniae* isolates from the intensive care units in which percentages have increased from 12% to 15%.[105]

The overall percentage of (gradient strip test-confirmed) carbapenem non-susceptible *E. coli* and *K. pneumoniae* in 2021 was only 0.04% for *E. coli* and 0.36% for *K. pneumoniae*.[105] This percentage has been fluctuating in the previous years for *E. coli* (increase from 0.04% in 2017 to 0.07% in 2019 and decrease to 0.05% in 2020) and has slightly decreased for *K. pneumoniae* (from 0.50% in 2017-2019 to 0.36% in 2021). In total, 242 carbapenemase-producing Enterobacterales (CPE) from 209 patients were sent in as part of the CPE surveillance in 2021. This number has been fluctuating in the previous years (increase from 244 in 2017 to 397 in 2019 and decrease to 225 in 2020). The decrease compared to 2019 can most likely be explained by the decreased travelling and a reduction in regular healthcare during the COVID-19 pandemic. The carbapenemase genes that were observed the most in the Netherlands in 2021 were $bla_{\text{OXA-48}}$ and bla_{OXA48}-like genes (42% of CPE isolates).[105]

The percentage of diagnostic *S. aureus* isolates that was identified as MRSA was 2% in 2021. This is similar to previous years. In total, 2,577 *S. aureus* isolates were send in as part of the national MRSA surveillance in 2021. When only the first isolate per person was included, this was similar to 2020 with 2,379 isolates but lower than 2017-2019 (3,152-3,309). This decrease may also be due to the COVID-19 pandemic. The multiple-locus variable number of tandem repeat analysis (MLVA) complex that was the most common in 2021 was MC0398 (23%), which is also called livestock-associated MRSA.[105]

The percentage of VRE in diagnostic isolates is low, around 0.3%. However, several VRE outbreaks are reported each year, with eight in 2021, five in 2020 and 19 in 2019.[105] In 2022, a consultation among national experts took place at the RIVM to discuss the Dutch VRE policies. More concrete plans are expected to be made in 2023.

Also in the Netherlands, AMR is regarded a One Health issue. Fortunately, using the data of Maran, the use of antimicrobials in animals has been decreasing since more than ten years.[105] This has led to the reduction of AMR isolates in most livestock species.

The role of the gut microbiota in colonisation with multidrug-resistant bacteria

Similar as for patients with *C. difficile* infections, patients colonised with MDR bacteria have frequently used antibiotics. The use of antibiotics influences the resistome of commensal microorganisms or potential pathogenic bacteria in the gut by survival and outgrowth of resistant strains, even for longer periods in the absence of this selective pressure.[157,158] The use of antibiotics and other factors such as specific diets, presence of comorbidity or use of other medication, could lead to a disturbed gut microbiota. This may result in decreased colonisation resistance against MDRO by e.g. an altered bile acid metabolism, an altered epithelial barrier or decreased bacteriocin production.[46,159] In the absence of perseverance of microbiota-disturbing factors, the microbiota has the ability to return to

its pre-disturbed state after some time.[46] A disturbed microbiota results in an increased susceptibility to colonisation with potentially pathogenic bacteria and subsequent development of infections.[159] For instance, a mouse study on VRE showed that a four-member consortium of commensal bacteria containing *Bacteroides sartorii*, *Parabacteroides distasonis*, and Clostridium cluster XIVa members *Clostridium bolteae* and *Blautia producta*, restored colonisation resistance against VRE.[160] A disturbed microbiota provides favourable conditions for colonisation with MDRO and it could act as a reservoir for horizontal AMR gene transfer within and between gut bacterial species.[159]

The gut microbiota can provide colonisation resistance by direct or indirect inhibition of potentially pathogenic bacteria or MDR bacteria (Figure 8).

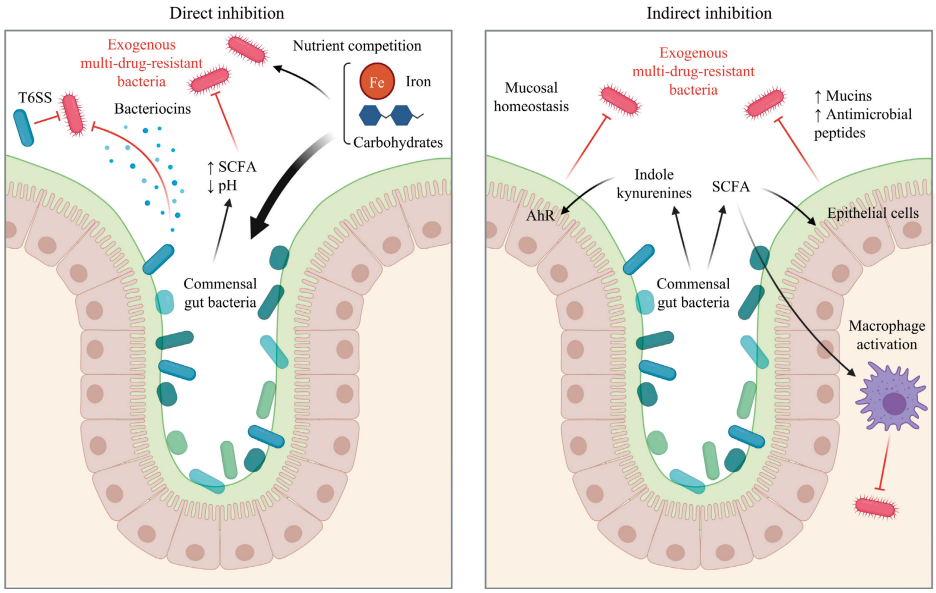


Figure 8. Direct and indirect mechanisms by which the gut microbiota can mediate colonisation resistance against multidrug-resistant bacteria.

The gut microbiota can provide colonisation resistance by direct inhibition (such as nutrient competition, the production of short-chain fatty acids or bacteriocins or conversion of primary bile acids) or indirect inhibition (by stimulating the immune system and the intestinal epithelium) of potentially pathogenic bacteria or MDR bacteria.

Abbreviations: AhR: aryl hydrocarbon receptor, SCFA: short-chain fatty acids, T6SS: type VI secretion system.

Reprinted from le Guern R, *et al.*, 2021, (p.50)[161] with permission from Elsevier. Figure created with BioRender.com. Adapted with permission.

It has been suggested that some commensal bacterial species in the gut prevent potentially pathogenic bacteria from becoming antibiotic-resistant. These commensals may influence the capability to benefit from chromosomal mutations and may inhibit the uptake of AMR genes by constraining plasmid transfer.[162] In general, *Bacteroides, Blautia,* Lachnospiraceae, *Prevotella,* and Ruminococcaceae are decreased in patients that are colonised with MDRO, suggesting they may play a protective role against MDRO colonisation.[46] Some commensal anaerobic bacteria produce butyrate, which is important in the regulation of intestinal epithelial homeostasis and immune functions.[163,164] A decrease of butyrate can lead to outgrowth of aerobic potentially pathogenic bacteria.[165] *Blautia producta* inhibits VRE colonisation by secreting a lantibiotic, a lanthionine-containing antimicrobial peptide, that inhibits the growth of VRE.[166] Another study showed that the colonisation resistance of the gut microbiota against *K. pneumoniae* is enhanced after a previous *K. pneumoniae* infection by the production of sulfide through taurine conversion pathways, decreasing cellular respiration, which is important for host invasion for several pathogens.[167] Interestingly, some strains of the commensal *Klebsiella oxytoca* can compete with pathogenic MDR *K. pneumoniae* in the murine gut by carbohydrate competition, but not without support from other members of the gut microbiota.[168] Commensal bacteria that produce beta-lactamase enzymes could also protect pathogens against antibiotics, thereby promoting the intestinal dissemination of MDRO.[169]

Araos *et al*[170] found reduced alpha diversity (Shannon) and a different composition of the gut microbiota in hospitalised patients with intestinal MDRO colonisation compared to controls, even after correction for comorbidity, previous use of antibiotics and prior stay in healthcare facilities. MDRO colonisation was associated with increased abundance of other multidrug resistance associated genes. Reduced alpha diversity and a different gut microbiota composition was also observed by Korach-Rechtman *et al.*,[171] who compared hospitalised patients colonised with carbapenem-resistant Enterobacterales (CRE) with hospitalised control patients and healthy controls. The CRE carriers had an increased abundance of several members of the Enterobacterales order and reduced abundance of several beneficial anaerobic commensals. Peled *et al.* found that patients with a more diverse gut microbiota had increased survival rates and decreased MDRO colonisation after hematopoietic stem cell transplantation. However, the matched case-control study of Ducarmon *et al.* found no difference in diversity parameters or in relative abundance of the gut microbiota between subjects that had an ESBL-producing *E. coli* compared to matched ESBL-negative subjects.[172]

Little is known about the association between the presence of *mcr* genes and the gut microbiota. One study[173] found that 2% of 214,095 publicly available metagenomes contained reads aligning to *mcr* genes, with *mcr-1* and *mcr-9* being the most frequently found. Another study[174] found 2,079 *mcr*-like genes in 2,046 genomes (1,880 metagenomic assembled genomes and 166 complete genomes from isolates) from the gut microbiota,

of which 215 were identified in plasmidial contigs. Most *mcr*-like genes were observed in the genera *Suterella* and *Parasuterella,* which include mostly commensals, but *mcr* genes were also identified in potential pathogens, such as *Escherichia coli, Vibrio species* and *Campylobacter hominis.* Furthermore, in these 2,046 genomes, 22,746 AMR genes belonging to 21 different antibiotic classes were found, suggesting a multidrug-resistant potential of these gut microbiomes.

FMT as treatment strategy for the colonisation with multidrug-resistant bacteria

Colonisation of the gut with MDRO can result in spread of the MDRO and invasive infections with high morbidity and mortality.[175-178] Specific antibiotic therapy may not be sufficient to achieve sustained cure, i.e. the MDRO may not be eradicated from the intestinal tract, resulting in recurrent infections and continuous spread.[179,180]

Data on other possible indications for FMT than CDI (e.g., hepatic encephalopathy, autism spectrum disorder, ulcerative colitis) are becoming available in experimental settings, but results frequently do not meet the high expectations.[181,182] FMT has also been suggested as treatment strategy for the eradication of MDRO from the gut.[183,184] In patients with recurrent CDI, the number of antibiotic resistance genes present in the faeces decreased after FMT.[83,185,186] Several studies examining the efficacy of FMT in patients with intestinal MDRO colonisation have been published, but no conclusions can be drawn due to the low quality of and considerable heterogeneity between studies.[183,184] Decolonisation rates varied greatly among these studies, ranging from 20 to 100% for patients treated with FMT and 10 to 66% for controls. This includes mostly case reports/series and cohort studies[183,184] and only one randomised controlled clinical trial (RCT).[187] Furthermore, sample sizes were small and the studies were heterogeneous regarding decolonisation definition, patient population, MDRO type, administration route, the number of FMT administrations, pre- and/or post-FMT treatment (antibiotics, bowel lavage, proton-pump inhibitor) and the duration of follow-up resulting in difficulties in comparing the results. In one frequently cited RCT,[187] thirty-nine immunocompetent carriers of ESBL- (ESBL-E) or CPE were randomised to either a five days course of oral colistin and neomycin followed by FMT (capsules or nasogastric approach) or no specific intervention. No significant difference in decolonisation rates between the two groups was found, but the planned sample size was not reached, different administration routes were used and there was no control group with only antibiotics.

Another important factor limiting the comparability and reliability of these studies is the phenomenon of spontaneous decolonisation. A systematic review and meta-analysis[188] showed that ESBL-E/CRE colonisation rates in health care settings decreased spontaneously to 76.7% at one month follow-up, 75.2% at three months follow-up, 55.3% at six months follow-up and further to 35.2% at 12 months follow-up. In the

community (mostly traveller studies), this was for ESBL-E 52.3%, 52.5%,19.2% and 25.4% at one, three, six and twelve months follow-up respectively. In another systematic review examining spontaneous intestinal/rectal decolonisation of VRE in hospitalised patients or long-term care facility residents, 80% of patients lost its VRE after 40 weeks, although not all decolonisation was confirmed with three separate swabs.[189] In a study with healthy subjects of the general population,[190] 12.4% of negative subjects acquired ESBL-producing *E. coli* or *K. pneumoniae* during a study period of 8 months. The duration of colonisation can vary greatly. E.g. for ESBL-producing microorganisms this varies per bacterial (sub)species and per ESBL enzyme.[191,192] Kantele *et al.*[193] demonstrated the potential transient nature of colonisation since they found that travellers could also carry ESBL-producing Enterobacterales for only a single day or a few days. Interestingly, the colonisation duration for ESBL-producing *E. coli* sequence type (ST) 131 is significantly longer compared to other STs, with a half-life of 13 months for ST131 versus two to three months for other STs.[194] These data underline the importance of including a control group in future studies.

Unfortunately, only seven studies assessing the efficacy of FMT in MDRO decolonisation included a control group.[187,195-200] Of the studies with a control group, four studies reported increased clearance of MDRO after FMT compared to the controls,[195-198] two studies reported only a minor non-significant improvement compared to controls,[187,199] and in one study the decolonisation rate of the control group was not reported.[200] Importantly, in the two studies with a control group that reported MDRO infection rates (one of which reported a minor non-significant improvement in MDRO colonisation and one reported no decolonisation rate for the control group), MDRO infections were clearly less frequently observed in the FMT group. Importantly, several other published studies have found reduced UTIs after FMT in patients with recurrent UTIs.[179,201-207] Furthermore, in a prospective cohort study examining the incidence of bloodstream infections (BSIs) in recurrent CDI patients treated with either FMT or antibiotics, less BSIs were observed in the FMT-group compared to the antibiotic group.[208] These studies suggest that FMT may potentially serve as a treatment strategy against recurrent MDRO infections originating from the gut without the necessity of complete eradication. FMT may inhibit the outgrowth of potential pathogens by restoring the gut microbiota composition. Furthermore, the decreased intestinal permeability established by treating CDI may prevent Gram-negative bacteria from entering the bloodstream. Other potential explanations for the decrease in BSIs and UTIs may be that FMT could reduce intestinal inflammation and the abundance of certain potentially pathogenic taxa, such as Enterobacterales.[183]

Parkinson's disease and the role of the gut microbiota

Epidemiological and clinical characteristics of Parkinson's disease

Parkinson's disease (PD) is a progressive neurodegenerative disease. The main motor symptoms of PD patients include tremor, bradykinesia, rigidity, and postural instability, but several other motor symptoms can be observed.[209,210] Non-motor symptoms include gastrointestinal complaints, cognitive dysfunction, sleep disturbances, fatigue, psychosis, hallucinations, mood disorders (including depression, anxiety, and apathy/abulia), autonomic dysfunction, pain and other sensory disturbances, olfactory dysfunction, and dermatological problems.[211] Disease progression in PD is variable. Approximately 77% have a poor outcome (as defined by death, dementia, postural instability) at 10 years after diagnosis.[212] Mortality in PD patients is moderately increased compared to age-matched controls, with a pooled mortality ratio of approximately 1.5.[213] The main therapy for PD is levodopa, a dopamine precursor that can pass the blood-brain barrier in contrast to dopamine itself. This is frequently combined with a decarboxylase inhibitor, which inhibits the peripheral conversion of levodopa into dopamine.

The worldwide prevalence of PD is estimated to be 0.3% in the general population that is aged 40 years and older.[214] The estimated global number of patients with PD increased from 6 million in 2016 to 8.5 million people in 2019,[215] which could only partially be explained by the increase in the number of older people worldwide. The age-standardised incidence rate of PD was 13.43 per 100,000 persons in 2019.[216] In 2020, there were about 36,300 PD patients in the Netherlands.[217] Several potential risk factors for PD are described. Many include environmental and other potentially modifiable risk factors, such as exposure to pesticides. However, evidence is frequently contradicting. Older age and a family history of PD are the only consistent risk factors, whereas cigarette smoking is a consistent protective factor. The male gender is also a frequently described risk factor.[218]

PD is characterised by the degeneration of dopaminergic and other neurons in the central nervous system (CNS), enteric nervous system (ENS) and peripheral autonomic nervous system, and the presence of Lewy bodies and Lewy neuritis in affected neurons[219]. An important factor in the aetiology of PD may be the misfolding and aggregation of the protein alpha-synuclein (αSyn), a major component of Lewy-bodies.[220] Typical abnormalities in the brains of PD patients include depigmentation, neuronal loss, and gliosis, particularly in the substantia nigra pars compacta (SNc) and in the pontine locus ceruleus.[218] However, the aetiology and pathogenesis of PD are still mostly unknown.

The role of the gut microbiota in Parkinson's disease

The gut can modulate the central nervous system through production of a variety of metabolites, neuroactive substances and gut hormones, that can travel to the brain via the

enteric nervous system, vagus nerve, circulatory system, or immune system. On the other hand, the brain also influences the function of the gut via the hypothalamic-pituitary-adrenal axis and the autonomic nervous system. Collectively, these pathways are called the (microbiota-)gut-brain axis.[221]

PD patients frequently have GI symptoms (including constipation and delayed transit) and these often precede the onset of motor symptoms, thus representing the first clinical manifestation of PD.[222,223] This suggests that the disease might be initiated in the gut. Concomitantly, several studies have demonstrated that alpha-synucleinopathy is present in the ENS and vagal nerves in an early phase of PD.[224-229] This led to the hypothesis that the disease may start in the gut, with a neurotrophic pathogen that is transported from the GI tract to the CNS by way of retrograde axonal and transneuronal transport through the vagal nerve.[224] This neurotrophic pathogen might consist of misfolded αSyn molecular fragments.[224,230] The hypothesis is supported by studies suggesting that αSyn can spread from neuron to neuron[231] and that αSyn forms could be transported from the gut to the brain in mouse models.[232-234] It is further suggested that aggregation of αSyn in the brain and possibly the gut of PD patients is a consequence of inflammation-induced oxidative stress.[235-237] Interestingly, PD patients have more signs of inflammation of the colon compared to healthy controls as measured by mRNA expression levels of pro-inflammatory cytokines and glial markers in colonic biopsies.[238] This finding suggests that there might be a role for intestinal inflammation in the initiation and/or the progression of PD.

It has been hypothesised that the gut microbiota and their metabolites play an important role in the pathogenesis and course of PD. A potential beneficial effect of providing faeces of healthy donors via FMT on the course of PD is shown in several mouse studies.[239-241] Several recent studies indicate that the gut microbiota and their metabolic products in PD patients are different from healthy individuals,[236,242-249] and the alpha-diversity (within-subject diversity) is higher than that of controls[249] (although there is some inconsistency in the results between studies).[244,247,248,250] Other important findings are an overall more pro-inflammatory and less anti-inflammatory gut microbiota composition in PD patients,[249,251] with more opportunistic pathogens, dysregulated neuroactive signaling, toxic metabolites,[252] toxicants[253] and immunogenic bacterial components, such as LPS.[236,252] There is an increase in genes involved in degradation of the neuroprotective molecules trehalose and nicotinamide,[252] and a metabolomics study indeed found a decrease in nicotinamide.[253] Furthermore, PD patients have increased intestinal permeability compared to healthy controls.[237,254] The "leaky gut" in PD patients may allow gut microbes or bacterial toxins, such as LPS, to enter the bloodstream, leading to blood brain barrier destruction and systemic and neuro-inflammation.[255] The most consistent gut microbiota alterations compared to controls in published studies are increased abundance of the genera *Lactobacillus*, *Bifidobacterium and Akkermansia* and

decreased abundance of bacteria belonging to the Lachnospiraceae family and the genus *Faecalibacterium*. The latter two are both important SCFA-producers, mainly involving butyrate.[249] Lower faecal levels of butyrate correlate with gait disorders, postural instability, depression and severity of motor symptoms (as measured by the Movement Disorders Society Unified Parkinson Disease Rating Scale - MDS-UPDRS - part III motor score) in PD patients.[255] With MDS-UPDRS, various aspects of PD can be evaluated, including motor- and non-motor experiences of daily living and motor complications.[256] Surprisingly, one study found lower levels of faecal butyrate but higher levels of plasma butyrate in PD patients.[257] Furthermore, administration of SCFA to PD animal models also gave conflicting results.[255]

One study found that *Prevotella* abundance is negatively associated with disease severity.[258] Furthermore, reduced abundance of the family Lachnospiraceae and increased abundance of the families Lactobacillaceae, Christensenellaceae and Ruminococcaceae and the order Enterobacterales has been found to be associated with cognitive impairment, motor symptoms and/or postural instability in PD patients.[244,259-261] Animal studies showed that colonisation with curli (amyloid proteins)-producing *E. coli* can promote αSyn aggregation in the gut and the brain.[262-264] Wallen *et al.*[252] found an increased abundance in gene-families related to curli in faeces of PD patients. Another study showed that hydrogen sulfide, produced by certain gut bacteria such as genera of the family Desulfovibrionaceae and Enterobacterales order, may induce PD by inducing αSyn aggregation.[265] Bacteria of the genus *Desulfovibrio*, which are hydrogen sulfide producers, are indeed present at higher levels in PD patients compared to healthy controls and are associated with PD severity.[266] Interestingly, a recent study[267] found an increased production of 2-hydroxypyridine (2-HP) in PD patients compared to controls, which was associated with the Archaeal species *Methanobrevibacter smithii*. 2-HP increased αSyn aggregation in a yeast model and in human induced pluripotent stem cell derived enteric neurons. Furthermore, when 2-HP was injected intrastriatally in a PD mouse model, αSyn aggregation, PD motor symptoms and striatal degeneration increased.[267] In contrast, αSyn aggregation may be attenuated by 3-(3-hydroxyphenyl) propionic acid (3-HPPA),[268] a gut microbiota fermentation product of dietary polyphenols and drugs, such as levodopa, which was found in lower levels in PD faeces compared to control faeces.[253]

Two other studies suggested that gut bacterial tyrosine decarboxylases can metabolise the frequently used PD medication levodopa to dopamine in PD patients without being susceptible for aromatic amino acid decarboxylase inhibitors, such as the frequently used carbidopa. Increased presence of gut bacterial tyrosine decarboxylases may thereby result in altered levodopa concentrations in the blood and, as a consequence, may potentially increase motor fluctuations in levodopa/carbidopa-treated PD patients as dopamine cannot cross the blood-brain barrier.[269,270]

All the above mentioned studies underline a possible role of gut bacteria in PD pathogenesis and/or the availability or absorption of PD medication. The microbiota-gut-brain axis in PD is visualised in Figure 9.

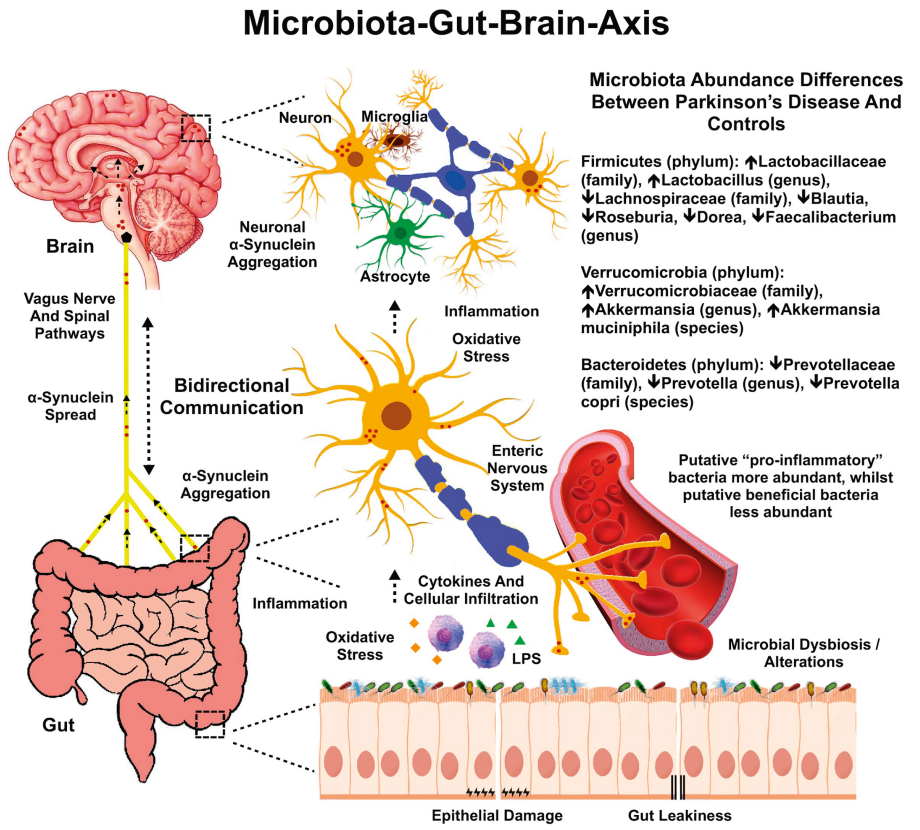


Figure 9. Assumed microbiota-gut-brain axis in Parkinson's disease.

This cartoon illustrates the hypothesis that an altered gut microbiota composition with increased pro-inflammatory bacteria induces intestinal inflammation and thereby oxidative stress with alpha-synuclein aggregation in the enteric nervous system; Alpha-synuclein then spreads through the vagal nerve to the brain. This is accompanied by increased gut permeability, which results in systemic inflammation and blood brain barrier disruption, resulting in neuroinflammation.
Abbreviations: LPS: lipopolysaccharides.
Retrieved unchanged from Lubomski M, *et al.*[271], reproduced with permission from Springer Nature.

FMT as treatment strategy for Parkinson's disease

At the moment, only symptomatic treatment for PD is available. Since there are no treatments available that cure PD or slow down the progression, the development of a new treatment strategy is crucial. The potential role of the gut microbiota in disease pathogenesis and/or availability or absorption of PD medication suggest that microbiota interventions, such as probiotics, prebiotics, synbiotics, dietary interventions, live biotherapeutic products, antibiotics or FMT, may be useful as treatment strategy.

One RCT showed an improvement in MDS-UPDRS score when PD medication was combined with probiotics.[272] However, whether there were any changes in PD medication during the study in the two groups was not mentioned. Another RCT showed improvement of sleep quality, anxiety, and GI symptoms after probiotics, but no improvement of UPDRS-III score or cognitive function compared to placebo.[273] An open-label, single-arm, baseline-controlled pilot trial found similar results, but this did not include randomisation with placebo treatment and blinding.[274] Most studies only examined the effect of probiotics on constipation and found an increase in defaecation frequency after probiotics. However, heterogeneity was high and the quality of evidence low.[275,276] Evidence on the usefulness of prebiotics,[239,277-282] synbiotics[283,284] and live biotherapeutic products[285,286] as treatment for PD is rather scarce.[287,288] Furthermore, it is still uncertain which specific species are beneficial for PD and which might potentially cause deterioration of PD. Important for the use of prebiotics is that, as mentioned before, evidence on the effect of SCFAs on PD shows contrasting results.[255,257] There are also studies that suggest a role for diet in PD, although results are inconsistent.[289] Interestingly, the first phase 1 trial with live biotherapeutic products was announced to start mid-2022.[290] The possibility to genetically engineer live biotherapeutic products makes them interesting candidates. Furthermore, there is some evidence suggesting that several antibiotics, including rifampicin, tetracyclines and beta-lactams, may have neuroprotective effects in PD patients (e.g. by decreasing neuroinflammation).[291] The chronic use of antibiotics in the treatment of PD may not be preferable, as it causes other kind of imbalances of the gut microbiota and may trigger the development of antimicrobial resistance.

Available literature suggests that FMT may be an option that not only improves PD symptoms and/or absorption and efficacy of PD medication but could potentially also influence the disease course. However, it is uncertain whether FMT is safe in this population due to the frequent swallowing problems, delayed gastric emptying and decreased GI motility in PD patients. Furthermore, it is unclear what would be the most effective administration route, number of FMTs, pre-treatment and donor or donor microbiota characteristics. The potential beneficial effect of FMT on the course of PD is shown in several mouse studies.[239-241] Sampson *et al.*[239] showed the importance of the gut microbiota in the development of motor symptoms in a PD mouse model with overexpression of αSyn (ASO), concluding that gut bacteria are necessary to induce

motor symptoms, alpha-synucleinopathy and neuro-inflammation. In this study, germ-free (GF) ASO mice showed less motor symptoms compared to specific-pathogen-free (SPF) ASO mice. When ASO mice received an FMT with faeces from human PD patients, motor symptoms increased, compared to mice that received an FMT with faeces from healthy human donors. The study suggests that FMT with faeces from healthy donors beneficially influences the course of PD. Meng-Fei Sun *et al.*[240] used a 1-methyl-4-fenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD mouse model and showed that mice that received a MPTP-injection had a better motor function after FMT with faeces of healthy mice, compared to MPTP-injected mice that received no FMT. Furthermore, healthy mice that received faeces from Parkinson mice performed worse compared to controls and in the traction test they performed even comparable to MPTP-injected mice. Zhou *et al.*[241] observed less motor function decline and attenuated loss of dopaminergic neurons in the substantia nigra in PD mice that received a fasting-mimicking diet (FMD) compared to *ad libitum*-fed PD mice. Furthermore, they observed a higher (more favourable) striatal dopamine and serotonin concentration in PD mice that had received faeces from FMD-fed control mice compared to phosphate-buffered solution (PBS)-gavaged or *ad libitum* microbiota-gavaged PD mice.

Given the above-mentioned evidence, FMT could be an interesting treatment for patients with PD. Only one case report[292] and three case series (15, 11, and 6 patients)[293-295] have been published so far reporting the results of FMT in PD patients. All series reported some improvement of motor and non-motor symptoms, including constipation. There were significant changes in the gut microbiota after FMT in the case report and the one case series (11 patients)[292,295] that reported gut microbiota analysis. Unfortunately, the case report and case series showed large variability of methods concerning pre-treatment, FMT administration route, follow-up and clinical evaluation. No results of RCTs on FMT in PD patients have been reported yet.

Outline of this thesis

This thesis is divided into three parts. The first part describes the epidemiology of infections with *Clostridioides difficile* and multidrug-resistant bacteria (MRSA and colistin-resistant Enterobacterales). The second part discusses the safety of FMT. The third part describes several neurological disorders as potential new indications for FMT.

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

Chapter 2 describes a retrospective sentinel surveillance study on the epidemiology of CDI in hospitalised patients during the coronavirus disease 2019 pandemic. The objectives of this study were to assess whether the CDI incidence and clinical and microbiological characteristics of CDI differed during the COVID-19 pandemic compared to previous years.

Chapter 3 discusses a community outbreak of impetigo by a meticillin-resistant *Staphylococcus aureus* strain, resistant to fusidic acid and with exfoliative toxin genes, in the eastern part of the Netherlands in 2019. It describes the results of an analysis of the extent and clinical, microbiological and genomic characteristics of this MRSA outbreak.

Chapter 4 reports on a prospective matched case-control study on the genomic epidemiology of colistin-resistant Enterobacterales from Dutch patients. This study describes the incidence and risk factors of patients colonised or infected with colistin-resistant *E. coli* or *K. pneumoniae* (COLR-EK) and the characterisation of these isolates.

Part 2: Infectious adverse events of faecal microbiota transplantation

Chapter 5 reports on a retrospective cohort study that discusses whether periodic screening of donor faeces with a quarantine period and screening after foreign travel is sufficient to prevent the presence of MDRO in faecal suspensions approved for use in faecal microbiota transplantation. Furthermore, the percentage of faeces donors that are colonised with MDRO in their gut is assessed and MDRO isolates from these donors are characterised.

Chapter 6 describes a case report of a patient that received FMT because of intestinal colonisation with an MDRO causing recurrent urinary tract infections, with transmission of an antibiotic susceptible *Escherichia coli* strain causing urinary tract infections as a result.

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

Chapter 7 provides a review of the potential new indications of FMT among neurological disorders, including PD. Publications on FMT in humans with, and animal models of, neurological disorders are discussed.

Chapter 8 describes a study protocol for a single centre, self-controlled, interventional, safety and feasibility donor-FMT pilot study in PD patients. 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.

Chapter 9 includes a summary of the most important findings of this thesis.

Chapter 10 provides a general discussion of the research presented in this thesis.

Chapter 11 includes recommendations for the future.

Chapter 12 contains a summary in the Dutch language.

References

1. Smits WK, Lyras D, Lacy DB, Wilcox MH, Kuijper EJ. Clostridium difficile infection. *Nat Rev Dis Primers* 2016; **2**: 16020.
2. 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).
3. European Centre for Disease Prevention and Control. Healthcare-associated infections: Clostridium difficile infections. In: ECDC. Annual epidemiological report for 2016. Stockholm: ECDC; 2018. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/AER_for_2016-C-difficile_0.pdf (accessed on the 21th of April 2022).
4. Rupnik M, Wilcox MH, Gerding DN. Clostridium difficile infection: new developments in epidemiology and pathogenesis. *Nat Rev Microbiol* 2009; **7**(7): 526-36.
5. Magill SS, Edwards JR, Bamberg W, et al. Multistate point-prevalence survey of health care-associated infections. *The New England journal of medicine* 2014; **370**(13): 1198-208.
6. Suetens C, Latour K, Kärki T, et al. Prevalence of healthcare-associated infections, estimated incidence and composite antimicrobial resistance index in acute care hospitals and long-term care facilities: results from two European point prevalence surveys, 2016 to 2017. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* 2018; **23**(46).
7. Ofori E, Ramai D, Dhawan M, Mustafa F, Gasperino J, Reddy M. Community-acquired Clostridium difficile: epidemiology, ribotype, risk factors, hospital and intensive care unit outcomes, and current and emerging therapies. *The Journal of hospital infection* 2018; **99**(4): 436-42.
8. Khanna S, Pardi DS. The growing incidence and severity of Clostridium difficile infection in inpatient and outpatient settings. *Expert review of gastroenterology & hepatology* 2010; **4**(4): 409-16.
9. Vendrik K.E.W., et al. Fourteenth Annual Report of the National Reference Laboratory for Clostridioides difficile and results of the sentinel surveillance May 2019 - Jan 2021. Available from: https://www.rivm.nl/documenten/annual-report-c-difficile-reference-laboratory-may-2019-jan-2021 (accessed on the 21th of April 2022).
10. Barbut F, Day N, Bouée S, et al. Toxigenic Clostridium difficile carriage in general practice: results of a laboratory-based cohort study. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2019; **25**(5): 588-94.
11. Goorhuis A, Van der Kooi T, Vaessen N, et al. Spread and epidemiology of Clostridium difficile polymerase chain reaction ribotype 027/toxinotype III in The Netherlands. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2007; **45**(6): 695-703.
12. Hensgens MP, Goorhuis A, Dekkers OM, van Benthem BH, Kuijper EJ. All-cause and disease-specific mortality in hospitalized patients with Clostridium difficile infection: a multicenter cohort study. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2013; **56**(8): 1108-16.

13. Goorhuis A. Editorial commentary: Clostridium difficile ribotype 027: an intrinsically virulent strain, but clinical virulence remains to be determined at the bedside. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2015; **61**(2): 242-3.

14. van Steenbergen J, Debast S, van Kregten E, van den Berg R, Notermans D, Kuijper E. Isolation of Clostridium difficile ribotype 027, toxinotype III in the Netherlands after increase in C. difficile-associated diarrhoea. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* 2005; **10**(7): E050714.1.

15. Kuijper EJ, van den Berg RJ, Debast S, et al. Clostridium difficile ribotype 027, toxinotype III, the Netherlands. *Emerging infectious diseases* 2006; **12**(5): 827-30.

16. Hensgens MP, Goorhuis A, Notermans DW, van Benthem BH, Kuijper EJ. Decrease of hypervirulent Clostridium difficile PCR ribotype 027 in the Netherlands. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* 2009; **14**(45).

17. Finn E, Andersson FL, Madin-Warburton M. Burden of Clostridioides difficile infection (CDI) - a systematic review of the epidemiology of primary and recurrent CDI. *BMC Infect Dis* 2021; **21**(1): 456.

18. Slimings C, Riley TV. Antibiotics and hospital-acquired Clostridium difficile infection: update of systematic review and meta-analysis. *The Journal of antimicrobial chemotherapy* 2014; **69**(4): 881-91.

19. Hensgens MP, Goorhuis A, Dekkers OM, Kuijper EJ. Time interval of increased risk for Clostridium difficile infection after exposure to antibiotics. *The Journal of antimicrobial chemotherapy* 2012; **67**(3): 742-8.

20. Stevens V, Dumyati G, Fine LS, Fisher SG, van Wijngaarden E. Cumulative antibiotic exposures over time and the risk of Clostridium difficile infection. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2011; **53**(1): 42-8.

21. van Rossen TM, Ooijevaar RE, Vandenbroucke-Grauls C, et al. Prognostic factors for severe and recurrent Clostridioides difficile infection: a systematic review. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2021.

22. Davies KA, Longshaw CM, Davis GL, et al. Underdiagnosis of Clostridium difficile across Europe: the European, multicentre, prospective, biannual, point-prevalence study of Clostridium difficile infection in hospitalised patients with diarrhoea (EUCLID). *The Lancet Infectious diseases* 2014; **14**(12): 1208-19.

23. Gateau C, Couturier J, Coia J, Barbut F. How to: diagnose infection caused by Clostridium difficile. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2018; **24**(5): 463-8.

24. Crobach MJ, Planche T, Eckert C, et al. European Society of Clinical Microbiology and Infectious Diseases: update of the diagnostic guidance document for Clostridium difficile infection. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 2016; **22 Suppl 4**: S63-81. https://doi.org/10.1016/j.cmi.2016.03.010.

25. Curry SR, Muto CA, Schlackman JL, et al. Use of multilocus variable number of tandem repeats analysis genotyping to determine the role of asymptomatic carriers in Clostridium difficile transmission. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2013; **57**(8): 1094-102.

26. Eyre DW, Cule ML, Wilson DJ, et al. Diverse sources of C. difficile infection identified on whole-genome sequencing. *The New England journal of medicine* 2013; **369**(13): 1195-205.

27. Werkgroep Infectie Preventie. Infectiepreventieve maatregelen bij Clostridium difficile. Available at: http://www.rivm.nl/dsresource?objectid=rivmp:260520&type=org&disposition=inline&ns_nc=1v.

28. Tschudin-Sutter S KE, Durovic A, Vehreschild MJGT, Barbut F, Eckert C, Fitzpatrick F, Hell M, Norèn T, O'Driscoll J, Coia J, Gastmeier P, von Müller L, Wilcox MH, Widmer AF. Guidance document for prevention of Clostridium difficile infection in acute healthcare settings. *Clinical Microbiology and Infection* 2018; **24**(10): 1051-4.

29. Sethi AK, Al-Nassir WN, Nerandzic MM, Bobulsky GS, Donskey CJ. Persistence of skin contamination and environmental shedding of Clostridium difficile during and after treatment of C. difficile infection. *Infection control and hospital epidemiology* 2010; **31**(1): 21-7.

30. Khanafer N, Voirin N, Barbut F, Kuijper E, Vanhems P. Hospital management of Clostridium difficile infection: a review of the literature. *The Journal of hospital infection* 2015; **90**(2): 91-101.

31. European Centre for Disease Prevention and Control. Antimicrobial consumption in the EU/EEA (ESAC-Net) - Annual Epidemiological Report for 2020. Stockholm: ECDC; 2020. https://www.ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-consumption-europe-2020#:~:text=Antimicrobial%20consumption%20is%20expressed%20as,range%3A%208.5%E2%80%9328.9 (Accessed on Nov, 18 2021).

32. van Prehn J, Reigadas E, Vogelzang EH, et al. European Society of Clinical Microbiology and Infectious Diseases: 2021 update on the treatment guidance document for Clostridioides difficile infection in adults. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2021; **27 Suppl 2**: S1-s21.

33. Johnson S, Lavergne V, Skinner AM, et al. Clinical Practice Guideline by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA): 2021 Focused Update Guidelines on Management of Clostridioides difficile Infection in Adults. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2021; **73**(5): 755-7.

34. Hopkins RJ, Wilson RB. Treatment of recurrent Clostridium difficile colitis: a narrative review. *Gastroenterol Rep (Oxf)* 2018; **6**(1): 21-8.

35. Guery B, Galperine T, Barbut F. Clostridioides difficile: diagnosis and treatments. *Bmj* 2019; **366**: l4609.

36. Beinortas T, Burr NE, Wilcox MH, Subramanian V. Comparative efficacy of treatments for Clostridium difficile infection: a systematic review and network meta-analysis. *The Lancet Infectious diseases*; **18**: 1035-44.

37. Wilcox MH, Gerding DN, Poxton IR, et al. Bezlotoxumab for Prevention of Recurrent Clostridium difficile Infection. *The New England journal of medicine* 2017; **376**(4): 305-17.

38. 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.

39. O'Grady K, Knight DR, Riley TV. Antimicrobial resistance in Clostridioides difficile. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology* 2021; **40**(12): 2459-78.

40. Boekhoud IM, Hornung BVH, Sevilla E, et al. Plasmid-mediated metronidazole resistance in Clostridioides difficile. *Nature communications* 2020; **11**(1): 598.

41. Saha S, Kapoor S, Tariq R, et al. Increasing antibiotic resistance in Clostridioides difficile: A systematic review and meta-analysis. *Anaerobe* 2019; **58**: 35-46.

42. Freeman J, Vernon J, Pilling S, et al. Five-year Pan-European, longitudinal surveillance of Clostridium difficile ribotype prevalence and antimicrobial resistance: the extended ClosER study. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology* 2020; **39**(1): 169-77.

43. Marchesi JR, Ravel J. The vocabulary of microbiome research: a proposal. *Microbiome* 2015; **3**: 31.

44. Maier L, Typas A. Systematically investigating the impact of medication on the gut microbiome. *Curr Opin Microbiol* 2017; **39**: 128-35.

45. Vasilescu IM, Chifiriuc MC, Pircalabioru GG, et al. Gut Dysbiosis and Clostridioides difficile Infection in Neonates and Adults. *Frontiers in microbiology* 2021; **12**: 651081.

46. Isles NS, Mu A, Kwong JC, Howden BP, Stinear TP. Gut microbiome signatures and host colonization with multidrug-resistant bacteria. *Trends Microbiol* 2022.

47. Nel Van Zyl K, Matukane SR, Hamman BL, Whitelaw AC, Newton-Foot M. Effect of antibiotics on the human microbiome: a systematic review. *Int J Antimicrob Agents* 2022; **59**(2): 106502.

48. Antharam VC, Li EC, Ishmael A, et al. Intestinal dysbiosis and depletion of butyrogenic bacteria in Clostridium difficile infection and nosocomial diarrhea. *Journal of clinical microbiology* 2013; **51**(9): 2884-92.

49. Schubert AM, Rogers MA, Ring C, et al. Microbiome data distinguish patients with Clostridium difficile infection and non-C. difficile-associated diarrhea from healthy controls. *mBio* 2014; **5**(3): e01021-14.

50. Zhang L, Dong D, Jiang C, Li Z, Wang X, Peng Y. Insight into alteration of gut microbiota in Clostridium difficile infection and asymptomatic C. difficile colonization. *Anaerobe* 2015; **34**: 1-7.

51. Crobach MJT, Ducarmon QR, Terveer EM, et al. The Bacterial Gut Microbiota of Adult Patients Infected, Colonized or Noncolonized by Clostridioides difficile. *Microorganisms* 2020; **8**(5).

52. Kachrimanidou M, Tsintarakis E. Insights into the Role of Human Gut Microbiota in Clostridioides difficile Infection. *Microorganisms* 2020; **8**(2).

53. Chang JY, Antonopoulos DA, Kalra A, et al. Decreased diversity of the fecal Microbiome in recurrent Clostridium difficile-associated diarrhea. *The Journal of infectious diseases* 2008; **197**(3): 435-8.

54. Britton RA, Young VB. Interaction between the intestinal microbiota and host in Clostridium difficile colonization resistance. *Trends Microbiol* 2012; **20**(7): 313-9.

55. Crobach MJT, Vernon JJ, Loo VG, et al. Understanding Clostridium difficile Colonization. *Clin Microbiol Rev* 2018; **31**(2).

56. Baktash A, Terveer EM, Zwittink RD, et al. Mechanistic Insights in the Success of Fecal Microbiota Transplants for the Treatment of Clostridium difficile Infections. Frontiers in microbiology 2018; 9: 1242. https://doi.org/10.3389/fmicb.2018.01242

57. Fuentes S, van Nood E, Tims S, et al. Reset of a critically disturbed microbial ecosystem: faecal transplant in recurrent Clostridium difficile infection. *Isme j* 2014; **8**(8): 1621-33.

58. Rizzatti G, Lopetuso LR, Gibiino G, Binda C, Gasbarrini A. Proteobacteria: A Common Factor in Human Diseases. *Biomed Res Int* 2017; **2017**: 9351507.

59. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res* 2013; **54**(9): 2325-40.

60. Bibbò S, Lopetuso LR, Ianiro G, Di Rienzo T, Gasbarrini A, Cammarota G. Role of microbiota and innate immunity in recurrent Clostridium difficile infection. *J Immunol Res* 2014; **2014**: 462740.

61. Gupta P, Yakubov S, Tin K, et al. Does Alkaline Colonic pH Predispose to Clostridium difficile Infection? *South Med J* 2016; **109**(2): 91-6.

62. Liu H, Wang J, He T, et al. Butyrate: A Double-Edged Sword for Health? *Adv Nutr* 2018; **9**(1): 21-9.

63. Wells JE, Hylemon PB. Identification and characterization of a bile acid 7alpha-dehydroxylation operon in Clostridium sp. strain TO-931, a highly active 7alpha-dehydroxylating strain isolated from human feces. *Applied and environmental microbiology* 2000; **66**(3): 1107-13.

64. Theriot CM, Bowman AA, Young VB. Antibiotic-Induced Alterations of the Gut Microbiota Alter Secondary Bile Acid Production and Allow for Clostridium difficile Spore Germination and Outgrowth in the Large Intestine. *mSphere* 2016; **1**(1).

65. Allegretti JR, Kearney S, Li N, et al. Recurrent Clostridium difficile infection associates with distinct bile acid and microbiome profiles. *Alimentary pharmacology & therapeutics* 2016; **43**(11): 1142-53.

66. Buffie CG, Bucci V, Stein RR, et al. Precision microbiome reconstitution restores bile acid mediated resistance to Clostridium difficile. *Nature* 2015; **517**(7533): 205-8.

67. Reed AD, Nethery MA, Stewart A, Barrangou R, Theriot CM. Strain-Dependent Inhibition of Clostridioides difficile by Commensal Clostridia Carrying the Bile Acid-Inducible (bai) Operon. *J Bacteriol* 2020; **202**(11).

68. Amrane S, Bachar D, Lagier JC, Raoult D. Clostridium scindens Is Present in the Gut Microbiota during Clostridium difficile Infection: a Metagenomic and Culturomic Analysis. *Journal of clinical microbiology* 2018; **56**(5).

69. Expression of Concern for Amrane et al., "Clostridium scindens Is Present in the Gut Microbiota during Clostridium difficile Infection: a Metagenomic and Culturomic Analysis". *Journal of clinical microbiology* 2022: e0107622.

70. Francis MB, Allen CA, Shrestha R, Sorg JA. Bile acid recognition by the Clostridium difficile germinant receptor, CspC, is important for establishing infection. *PLoS pathogens* 2013; **9**(5): e1003356.

71. Goldenberg JZ, Yap C, Lytvyn L, et al. Probiotics for the prevention of Clostridium difficile-associated diarrhea in adults and children. *The Cochrane database of systematic reviews* 2017; **12**(12): Cd006095.

72. Khanna S, Assi M, Lee C, et al. Efficacy and Safety of RBX2660 in PUNCH CD3, a Phase III, Randomized, Double-Blind, Placebo-Controlled Trial with a Bayesian Primary Analysis for the Prevention of Recurrent Clostridioides difficile Infection. *Drugs* 2022: 1-12.

73. Feuerstadt P, Louie TJ, Lashner B, et al. SER-109, an Oral Microbiome Therapy for Recurrent Clostridioides difficile Infection. *The New England journal of medicine* 2022; **386**(3): 220-9.

74. Dsouza M, Menon R, Crossette E, et al. Colonization of the live biotherapeutic product VE303 and modulation of the microbiota and metabolites in healthy volunteers. *Cell Host Microbe* 2022; **30**(4): 583-98.e8.

75. Khanna S, Pardi DS, Jones C, Shannon WD, Gonzalez C, Blount K. RBX7455, a Non-frozen, Orally Administered Investigational Live Biotherapeutic, Is Safe, Effective, and Shifts Patients' Microbiomes in a Phase 1 Study for Recurrent Clostridioides difficile Infections. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2021; **73**(7): e1613-e20.

76. Allegretti JR, Kelly CR, Louie T, et al. An investigational oral microbiome Drug, CP101, for the prevention of recurrent C. difficile infection: a randomized, placebo-controlled, multi-center trial (PRISM3). American College of Gastroenterology Annual Meeting. 2020.

77. Song YN, Yang DY, Veldhuyzen van Zanten S, et al. Fecal Microbiota Transplantation for Severe or Fulminant Clostridioides difficile Infection: Systematic Review and Meta-analysis. *J Can Assoc Gastroenterol* 2022; **5**(1): e1-e11.

78. Tixier EN, Verheyen E, Luo Y, et al. Systematic Review with Meta-Analysis: Fecal Microbiota Transplantation for Severe or Fulminant Clostridioides difficile. *Digestive diseases and sciences* 2022; **67**(3): 978-88.

79. Rupawala AH, Gachette D, Bakhit M, Jimoh L, Kelly CR. Management of Severe and Severe/Complicated Clostridoides difficile Infection Using Sequential Fecal Microbiota Transplant by Retention Enema. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2021; **73**(4): 716-9.

80. Singh T, Bedi P, Bumrah K, et al. Fecal Microbiota Transplantation and Medical Therapy for Clostridium difficile Infection: Meta-analysis of Randomized Controlled Trials. *J Clin Gastroenterol* 2021.

81. Baunwall SMD, Lee MM, Eriksen MK, et al. Faecal microbiota transplantation for recurrent Clostridioides difficile infection: An updated systematic review and meta-analysis. *EClinicalMedicine* 2020; **29-30**: 100642.

82. 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.

83. Song Y, Garg S, Girotra M, et al. Microbiota dynamics in patients treated with fecal microbiota transplantation for recurrent Clostridium difficile infection. *PloS one* 2013; **8**(11): e81330.

84. Weingarden AR, Chen C, Bobr A, et al. Microbiota transplantation restores normal fecal bile acid composition in recurrent Clostridium difficile infection. *American journal of physiology Gastrointestinal and liver physiology* 2014; **306**(4): G310-9.

85. Seekatz AM, Aas J, Gessert CE, et al. Recovery of the gut microbiome following fecal microbiota transplantation. *mBio* 2014; **5**(3): e00893-14.

86. Ott SJ, Waetzig GH, Rehman A, et al. Efficacy of Sterile Fecal Filtrate Transfer for Treating Patients With Clostridium difficile Infection. *Gastroenterology* 2017; **152**(4): 799-811.e7.

87. 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.

88. Adamu BO, Lawley TD. Bacteriotherapy for the treatment of intestinal dysbiosis caused by Clostridium difficile infection. Curr Opin Microbiol 2013; **16**(5): 596-601. https://doi.org/10.1016/j.mib.2013.06.009.

89. Weingarden AR, Dosa PI, DeWinter E, et al. Changes in Colonic Bile Acid Composition following Fecal Microbiota Transplantation Are Sufficient to Control Clostridium difficile Germination and Growth. *PloS one* 2016; **11**(1): e0147210.

90. van Leeuwen PT, van der Peet JM, Bikker FJ, et al. Interspecies Interactions between Clostridium difficile and Candida albicans. *mSphere* 2016; **1**(6).

91. Zuo T, Wong SH, Cheung CP, et al. Gut fungal dysbiosis correlates with reduced efficacy of fecal microbiota transplantation in Clostridium difficile infection. *Nature communications* 2018; **9**(1): 3663.

92. Terveer EM, van Beurden YH, Goorhuis A, et al. How to: Establish and run a stool bank. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2017; **23**(12): 924-30.

93. Wang S, Xu M, Wang W, et al. Systematic Review: Adverse Events of Fecal Microbiota Transplantation. *PloS one* 2016; **11**(8): e0161174.

94. Terveer EM, Vendrik KE, Ooijevaar RE, et al. Faecal microbiota transplantation for Clostridioides difficile infection: Four years' experience of the Netherlands Donor Feces Bank. *United European Gastroenterol J* 2020: 2050640620957765.

95. Wilcox MH, McGovern BH, Hecht GA. The Efficacy and Safety of Fecal Microbiota Transplant for Recurrent Clostridium difficile Infection: Current Understanding and Gap Analysis. *Open forum infectious diseases* 2020; **7**(5): ofaa114.

96. Baxter M, Colville A. Adverse events in faecal microbiota transplant: a review of the literature. *J Hosp Infect* 2016; **92**(2): 117-27.

97. Michailidis L, Currier AC, Le M, Flomenhoft DR. Adverse events of fecal microbiota transplantation: a meta-analysis of high-quality studies. *Ann Gastroenterol* 2021; **34**(6): 802-14.

98. Saha S, Sehgal K, Singh S, Grover M, Pardi D, Khanna S. Postinfection Irritable Bowel Syndrome Following Clostridioides difficile Infection: A Systematic-review and Meta-analysis. *J Clin Gastroenterol* 2022; **56**(2): e84-e93.

99. Perler BK, Chen B, Phelps E, et al. Long-Term Efficacy and Safety of Fecal Microbiota Transplantation for Treatment of Recurrent Clostridioides difficile Infection. *J Clin Gastroenterol* 2020; **54**(8): 701-6.

100. WHO Regional Office for Europe/European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2022 - 2020 data. Available from: https://www.ecdc.europa.eu/en/publications-data/antimicrobial-resistance-surveillance-europe-2022-2020-data (Accessed on the 29th of April 2022).

101. Partridge SR, Kwong SM, Firth N, Jensen SO. Mobile Genetic Elements Associated with Antimicrobial Resistance. *Clin Microbiol Rev* 2018; **31**(4).

102. Peirano G, Pitout JDD. Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae: Update on Molecular Epidemiology and Treatment Options. *Drugs* 2019; **79**(14): 1529-41.

103. Aslam B, Khurshid M, Arshad MI, et al. Antibiotic Resistance: One Health One World Outlook. *Frontiers in cellular and infection microbiology* 2021; **11**: 771510.

104. Rice LB. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. *The Journal of infectious diseases* 2008; **197**(8): 1079-81.

105. Stichting Werkgroep Antibioticabeleid (SWAB), Centre for Infectious Disease Control from the National Institute for Public Health and the Environment (RIVM). NethMap 2022. Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands in 2021. Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2021. June, 2022. https://www.rivm.nl/publicaties/nethmap-2022-consumption-of-antimicrobial-agents (accessed on Nov 21, 2022).

106. Werkgroep Infectiepreventie. ziekehuizen: Bijzonder resistente micro-organismen (BRMO). december 2012, revision december 2017

107. Bush K, Bradford PA. β-Lactams and β-Lactamase Inhibitors: An Overview. *Cold Spring Harb Perspect Med* 2016; **6**(8).

108. Pitout JD, Laupland KB. Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. *The Lancet Infectious diseases* 2008; **8**(3): 159-66.

109. European Centre for Disease Prevention and Control. Antimicrobial resistance in the EU/EEA (EARS-Net) - Annual Epidemiological Report for 2021. Available from: https://www.ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-2021-data (Accessed on the 21th of November 2022).

110. Rodríguez-Guerrero E, Callejas-Rodelas JC, Navarro-Marí JM, Gutiérrez-Fernández J. Systematic Review of Plasmid AmpC Type Resistances in Escherichia coli and Klebsiella pneumoniae and Preliminary Proposal of a Simplified Screening Method for ampC. *Microorganisms* 2022; **10**(3).

111. Taggar G, Attiq Rheman M, Boerlin P, Diarra MS. Molecular Epidemiology of Carbapenemases in Enterobacteriales from Humans, Animals, Food and the Environment. *Antibiotics (Basel)* 2020; **9**(10).

112. Aurilio C, Sansone P, Barbarisi M, et al. Mechanisms of Action of Carbapenem Resistance. *Antibiotics (Basel)* 2022; **11**(3).

113. Nation RL, Li J. Polymyxins. In: Kucers' The Use of Antibiotics: A Clinical Review of Antibacterial, Antifungal, Antiparasitic, and Antiviral Drugs, 7th ed, Grayson ML, Cosgrove SE, Crowe SM, et al (Eds), CRC Press, Boca Raton 2018. p.1420.

114. Evans ME, Feola DJ, Rapp RP. Polymyxin B sulfate and colistin: old antibiotics for emerging multiresistant gram-negative bacteria. *Ann Pharmacother* 1999; **33**(9): 960-7.

115. Li J, Turnidge J, Milne R, Nation RL, Coulthard K. In vitro pharmacodynamic properties of colistin and colistin methanesulfonate against Pseudomonas aeruginosa isolates from patients with cystic fibrosis. *Antimicrobial agents and chemotherapy* 2001; **45**(3): 781-5.

116. Giacometti A, Cirioni O, Ghiselli R, et al. Antiendotoxin activity of antimicrobial peptides and glycopeptides. *J Chemother* 2003; **15**(2): 129-33.

117. Warren HS, Kania SA, Siber GR. Binding and neutralization of bacterial lipopolysaccharide by colistin nonapeptide. *Antimicrobial agents and chemotherapy* 1985; **28**(1): 107-12.

118. Nation RL, Li J. Colistin in the 21st century. *Curr Opin Infect Dis* 2009; **22**(6): 535-43.

119. World Organisation for Animal Health (OIE). OIE annual report on antimicrobial agents intended for use in animals, 6th report. 2022. Available from: https://www.woah.org/app/uploads/2022/06/a-sixth-annual-report-amu-final-1.pdf (accesed on the 26th of November 2022).

120. Catry B, Cavaleri M, Baptiste K, et al. Use of colistin-containing products within the European Union and European Economic Area (EU/EEA): development of resistance in animals and possible impact on human and animal health. *Int J Antimicrob Agents* 2015; **46**(3): 297-306.

121. Galani I, Karaiskos I, Karantani I, et al. Epidemiology and resistance phenotypes of carbapenemase-producing *Klebsiella pneumoniae* in Greece, 2014 to 2016. *Euro Surveill* 2018; **23**(31).

122. Monaco M, Giani T, Raffone M, et al. Colistin resistance superimposed to endemic carbapenem-resistant *Klebsiella pneumoniae*: a rapidly evolving problem in Italy, November 2013 to April 2014. *Euro Surveill* 2014; **19**(42).

123. Parisi SG, Bartolini A, Santacatterina E, et al. Prevalence of *Klebsiella pneumoniae* strains producing carbapenemases and increase of resistance to colistin in an Italian teaching hospital from January 2012 To December 2014. *BMC Infect Dis* 2015; **15**: 244.

124. Gogry FA, Siddiqui MT, Sultan I, Haq QMR. Current Update on Intrinsic and Acquired Colistin Resistance Mechanisms in Bacteria. *Front Med (Lausanne)* 2021; **8**: 677720.

125. Poirel L, Jayol A, Nordmann P. Polymyxins: Antibacterial Activity, Susceptibility Testing, and Resistance Mechanisms Encoded by Plasmids or Chromosomes. *Clin Microbiol Rev* 2017; **30**(2): 557-96.

126. Schwarz S, Johnson AP. Transferable resistance to colistin: a new but old threat. *J Antimicrob Chemother* 2016; **71**(8): 2066-70.

127. Liu YY, Wang Y, Walsh TR, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *The Lancet Infectious diseases* 2016; **16**(2): 161-8.

128. Xu Y, Wei W, Lei S, Lin J, Srinivas S, Feng Y. An Evolutionarily Conserved Mechanism for Intrinsic and Transferable Polymyxin Resistance. mBio 2018; **9**(2). https://doi.org/10.1128/mBio.02317-17.

129. Doijad SP, Gisch N, Frantz R, et al. Resolving colistin resistance and heteroresistance in Enterobacter species. *Nature communications* 2023; **14**(1): 140.

130. Shen Z, Wang Y, Shen Y, Shen J, Wu C. Early emergence of mcr-1 in Escherichia coli from food-producing animals. *The Lancet Infectious diseases* 2016; **16**(3): 293.

131. Wang R, van Dorp L, Shaw LP, et al. The global distribution and spread of the mobilized colistin resistance gene *mcr-1*. *Nat Commun* 2018; **9**(1): 1179.

132. Chen K, Chan EW, Xie M, Ye L, Dong N, Chen S. Widespread distribution of *mcr-1*-bearing bacteria in the ecosystem, 2015 to 2016. *Euro Surveill* 2017; **22**(39).

133. Giamarellou H. Epidemiology of infections caused by polymyxin-resistant pathogens. *Int J Antimicrob Agents* 2016; **48**(6): 614-21.

134. Grundmann H, Glasner C, Albiger B, et al. Occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study. *Lancet Infect Dis* 2017; **17**(2): 153-63.

135. European Centre for Disease Prevention and Control. Surveillance of antimicrobial resistance in Europe: Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net) 2016 (2017). Available from: https://www.ecdc.europa.eu/en/publications-data/antimicrobial-resistance-surveillance-europe-2016 (Accessed on the 4th of May 2022).

136. European Centre for Disease Prevention and Control. ECDC study protocol for genomic-based surveillance of carbapenemresistant and/or colistin-resistant Enterobacteriaceae at the EU level. Version 2.0 (2018)(available from: https://www.ecdc.europa.eu/en/publications-data/ecdc-study-protocol-genomic-based-surveillance-carbapenem-resistant-andor (accessed on the 4th of May 2022).

137. Gould D, Chamberlaine A. Staphylococcus aureus: a review of the literature. *J Clin Nurs* 1995; **4**(1): 5-12.

138. Wertheim HF, Melles DC, Vos MC, et al. The role of nasal carriage in Staphylococcus aureus infections. *The Lancet Infectious diseases* 2005; **5**(12): 751-62.

139. Williams RE, Jevons MP, Shooter RA, et al. Nasal staphylococci and sepsis in hospital patients. *Br Med J* 1959; **2**(5153): 658-62.

140. Lakhundi S, Zhang K. Methicillin-Resistant Staphylococcus aureus: Molecular Characterization, Evolution, and Epidemiology. *Clin Microbiol Rev* 2018; **31**(4).

141. Kirby WM. Extraction of a highly potent penicillin inactivator from penicillin resistant staphylococci. *Science (New York, NY)* 1944; **99**(2579): 452-3.

142. Jevons MP. "Celbenin"-resistant staphylococci. Br Med J 1961; 1:124–125. .

143. Hartman BJ, Tomasz A. Low-affinity penicillin-binding protein associated with beta-lactam resistance in Staphylococcus aureus. *J Bacteriol* 1984; **158**(2): 513-6.

144. Utsui Y, Yokota T. Role of an altered penicillin-binding protein in methicillin- and cephem-resistant Staphylococcus aureus. *Antimicrobial agents and chemotherapy* 1985; **28**(3): 397-403.

145. Katayama Y, Ito T, Hiramatsu K. A new class of genetic element, staphylococcus cassette chromosome mec, encodes methicillin resistance in Staphylococcus aureus. *Antimicrobial agents and chemotherapy* 2000; **44**(6): 1549-55.

146. Turner NA, Sharma-Kuinkel BK, Maskarinec SA, et al. Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research. *Nat Rev Microbiol* 2019; **17**(4): 203-18.

147. Bai AD, Lo CKL, Komorowski AS, et al. Staphylococcus aureus bacteraemia mortality: a systematic review and meta-analysis. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2022; **28**(8): 1076-84.

148. Athanassa Z, Siempos, II, Falagas ME. Impact of methicillin resistance on mortality in Staphylococcus aureus VAP: a systematic review. *Eur Respir J* 2008; **31**(3): 625-32.

149. Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible Staphylococcus aureus bacteremia: a meta-analysis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2003; **36**(1): 53-9.

150. Otto M. Community-associated MRSA: what makes them special? *Int J Med Microbiol* 2013; **303**(6-7): 324-30.

151. Joshi S, Shallal A, Zervos M. Vancomycin-Resistant Enterococci: Epidemiology, Infection Prevention, and Control. *Infect Dis Clin North Am* 2021; **35**(4): 953-68.

152. Olivier CN, Blake RK, Steed LL, Salgado CD. Risk of vancomycin-resistant Enterococcus (VRE) bloodstream infection among patients colonized with VRE. *Infection control and hospital epidemiology* 2008; **29**(5): 404-9.

153. Guffey AA, Loll PJ. Regulation of Resistance in Vancomycin-Resistant Enterococci: The VanRS Two-Component System. *Microorganisms* 2021; **9**(10).

154. Werner G, et al. Enterococcus faecium strains from bloodstream infections of German hospital patients revealed a preferred prevalence of ST117 and an increasing number of vanB-type VRE between 2011 and 2017. In: European Congress of Microbiology and Infectious Diseases, Madrid; 2018.

155. Sparo M, Delpech G, García Allende N. Impact on Public Health of the Spread of High-Level Resistance to Gentamicin and Vancomycin in Enterococci. *Frontiers in microbiology* 2018; **9**: 3073.

156. Cassini A, Högberg LD, Plachouras D, et al. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. *The Lancet Infectious diseases* 2019; **19**(1): 56-66.

157. Xu L, Surathu A, Raplee I, et al. The effect of antibiotics on the gut microbiome: a metagenomics analysis of microbial shift and gut antibiotic resistance in antibiotic treated mice. *BMC Genomics* 2020; **21**(1): 263.

158. Jernberg C, Löfmark S, Edlund C, Jansson JK. Long-term impacts of antibiotic exposure on the human intestinal microbiota. *Microbiology (Reading)* 2010; **156**(Pt 11): 3216-23.

159. Kim S, Covington A, Pamer EG. The intestinal microbiota: Antibiotics, colonization resistance, and enteric pathogens. *Immunol Rev* 2017; **279**(1): 90-105.

160. Caballero S, Kim S, Carter RA, et al. Cooperating Commensals Restore Colonization Resistance to Vancomycin-Resistant Enterococcus faecium. *Cell Host Microbe* 2017; **21**(5): 592-602.e4.

161. Le Guern R, Stabler S, Gosset P, et al. Colonization resistance against multi-drug-resistant bacteria: a narrative review. *The Journal of hospital infection* 2021; **118**: 50.

162. Baumgartner M, Bayer F, Pfrunder-Cardozo KR, Buckling A, Hall AR. Resident microbial communities inhibit growth and antibiotic-resistance evolution of Escherichia coli in human gut microbiome samples. *PLoS Biol* 2020; **18**(4): e3000465.

163. Sun Y, O'Riordan MX. Regulation of bacterial pathogenesis by intestinal short-chain Fatty acids. *Adv Appl Microbiol* 2013; **85**: 93-118.

164. Keith JW, Pamer EG. Enlisting commensal microbes to resist antibiotic-resistant pathogens. *The Journal of experimental medicine* 2019; **216**(1): 10-9.

165. Rivera-Chávez F, Zhang LF, Faber F, et al. Depletion of Butyrate-Producing Clostridia from the Gut Microbiota Drives an Aerobic Luminal Expansion of Salmonella. *Cell Host Microbe* 2016; **19**(4): 443-54.

166. Kim SG, Becattini S, Moody TU, et al. Microbiota-derived lantibiotic restores resistance against vancomycin-resistant Enterococcus. *Nature* 2019; **572**(7771): 665-9.

167. Stacy A, Andrade-Oliveira V, McCulloch JA, et al. Infection trains the host for microbiota-enhanced resistance to pathogens. *Cell* 2021; **184**(3): 615-27.e17.

168. Osbelt L, Wende M, Almási É, et al. Klebsiella oxytoca causes colonization resistance against multidrug-resistant K. pneumoniae in the gut via cooperative carbohydrate competition. *Cell Host Microbe* 2021; **29**(11): 1663-79.e7.

169. Gjonbalaj M, Keith JW, Do MH, Hohl TM, Pamer EG, Becattini S. Antibiotic Degradation by Commensal Microbes Shields Pathogens. *Infection and immunity* 2020; **88**(4).

170. Araos R, Montgomery V, Ugalde JA, Snyder GM, D'Agata EMC. Microbial Disruption Indices to Detect Colonization With Multidrug-Resistant Organisms. *Infection control and hospital epidemiology* 2017; **38**(11): 1312-8.

171. Korach-Rechtman H, Hreish M, Fried C, et al. Intestinal Dysbiosis in Carriers of Carbapenem-Resistant Enterobacteriaceae. *mSphere* 2020; **5**(2).

172. Ducarmon QR, Zwittink RD, Willems RPJ, et al. Gut colonisation by extended-spectrum β-lactamase-producing Escherichia coli and its association with the gut microbiome and metabolome in Dutch adults: a matched case-control study. *Lancet Microbe* 2022; **3**(6): e443-e51.

173. Martiny HM, Munk P, Brinch C, Szarvas J, Aarestrup FM, Petersen TN. Global Distribution of mcr Gene Variants in 214K Metagenomic Samples. *mSystems* 2022; **7**(2): e0010522.

174. Andrade BGN, Goris T, Afli H, Coutinho FH, Dávila AMR, Cuadrat RRC. Putative mobilized colistin resistance genes in the human gut microbiome. *BMC microbiology* 2021; **21**(1): 220.

175. Schwaber MJ, Navon-Venezia S, Kaye KS, Ben-Ami R, Schwartz D, Carmeli Y. Clinical and economic impact of bacteremia with extended- spectrum-beta-lactamase-producing Enterobacteriaceae. *Antimicrobial agents and chemotherapy* 2006; **50**(4): 1257-62.

176. Lautenbach E, Patel JB, Bilker WB, Edelstein PH, Fishman NO. Extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae: risk factors for infection and impact of resistance on outcomes. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2001; **32**(8): 1162-71.

177. Kang CI, Kim SH, Kim HB, et al. Pseudomonas aeruginosa bacteremia: risk factors for mortality and influence of delayed receipt of effective antimicrobial therapy on clinical outcome. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2003; **37**(6): 745-51.

178. Bilinski J, Robak K, Peric Z, et al. Impact of Gut Colonization by Antibiotic-Resistant Bacteria on the Outcomes of Allogeneic Hematopoietic Stem Cell Transplantation: A Retrospective, Single-Center Study. *Biol Blood Marrow Transplant* 2016; **22**(6): 1087-93.

179. Stalenhoef JE, Terveer EM, Knetsch CW, et al. Fecal Microbiota Transfer for Multidrug-Resistant Gram-Negatives: A Clinical Success Combined With Microbiological Failure. *Open forum infectious diseases* 2017; **4**(2): ofx047.

180. Kuijper EJ, Vendrik KEW, Vehreschild M. Manipulation of the microbiota to eradicate multidrug-resistant Enterobacteriaceae from the human intestinal tract. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2019; **25**(7): 786-9.

181. Green JE, Davis JA, Berk M, et al. Efficacy and safety of fecal microbiota transplantation for the treatment of diseases other than Clostridium difficile infection: a systematic review and meta-analysis. *Gut microbes* 2020; **12**(1): 1-25.

182. Vendrik KEW, Ooijevaar RE, de Jong PRC, et al. Fecal Microbiota Transplantation in Neurological Disorders. *Frontiers in cellular and infection microbiology* 2020; **10**: 98.

183. Bilsen MP, Lambregts MMC, van Prehn J, Kuijper EJ. Faecal microbiota replacement to eradicate antimicrobial resistant bacteria in the intestinal tract - a systematic review. *Curr Opin Gastroenterol* 2022; **38**(1): 15-25.

184. Dharmaratne P, Rahman N, Leung A, Ip M. Is there a role of faecal microbiota transplantation in reducing antibiotic resistance burden in gut? A systematic review and Meta-analysis. *Ann Med* 2021; **53**(1): 662-81.

185. Millan B, Park H, Hotte N, et al. Fecal Microbial Transplants Reduce Antibiotic-resistant Genes in Patients With Recurrent Clostridium difficile Infection. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2016; **62**(12): 1479-86.

186. Jouhten H, Mattila E, Arkkila P, Satokari R. Reduction of Antibiotic Resistance Genes in Intestinal Microbiota of Patients With Recurrent Clostridium difficile Infection After Fecal Microbiota Transplantation. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2016; **63**(5): 710-1.

187. Huttner BD, de Lastours V, Wassenberg M, et al. A 5-day course of oral antibiotics followed by faecal transplantation to eradicate carriage of multidrug-resistant Enterobacteriaceae: a randomized clinical trial. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2019; **25**(7): 830-8.

188. Bar-Yoseph H, Hussein K, Braun E, Paul M. Natural history and decolonization strategies for ESBL/carbapenem-resistant Enterobacteriaceae carriage: systematic review and meta-analysis. *The Journal of antimicrobial chemotherapy* 2016; **71**(10): 2729-39.

189. Shenoy ES, Paras ML, Noubary F, Walensky RP, Hooper DC. Natural history of colonization with methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococcus (VRE): a systematic review. *BMC Infect Dis* 2014; **14**: 177.

190. van den Bunt G, Fluit AC, Bootsma MCJ, et al. Dynamics of Intestinal Carriage of Extended-Spectrum Beta-lactamase-Producing Enterobacteriaceae in the Dutch General Population, 2014-2016. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2020; **71**(8): 1847-55.

191. Titelman E, Hasan CM, Iversen A, et al. Faecal carriage of extended-spectrum β-lactamase-producing Enterobacteriaceae is common 12 months after infection and is related to strain factors. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2014; **20**(8): O508-15.

192. Teunis PFM, Evers EG, Hengeveld PD, Dierikx CM, Wielders CCH, van Duijkeren E. Time to acquire and lose carriership of ESBL/pAmpC producing E. coli in humans in the Netherlands. *PloS one* 2018; **13**(3): e0193834.

193. Kantele A, Kuenzli E, Dunn SJ, et al. Dynamics of intestinal multidrug-resistant bacteria colonisation contracted by visitors to a high-endemic setting: a prospective, daily, real-time sampling study. *Lancet Microbe* 2021; **2**(4): e151-e8.

194. Overdevest I, Haverkate M, Veenemans J, et al. Prolonged colonisation with Escherichia coli O25:ST131 versus other extended-spectrum beta-lactamase-producing E. coli in a long-term care facility with high endemic level of rectal colonisation, the Netherlands, 2013 to 2014. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* 2016; **21**(42).

195. Lee JH, Shin JB, Ko WJ, et al. Efficacy and safety of fecal microbiota transplantation on clearance of multidrug resistance organism in multicomorbid patients: a prospective nonrandomized comparison trial. United Eur Gastroenterol J 2020; 8: (8 SUPPL): 499.

196. Seong H, Lee SK, Cheon JH, et al. Fecal Microbiota Transplantation for multidrug-resistant organism: Efficacy and Response prediction. *J Infect* 2020; **81**(5): 719-25.

197. Eysenbach L, Allegretti JR, Aroniadis O, et al. Clearance of vancomycin-resistant Enterococcus colonization with fecal microbiota transplantation among patients with recurrent Clostridium difficile infection. Open Forum Infect Dis.

198. Saïdani N, Lagier JC, Cassir N, et al. Faecal microbiota transplantation shortens the colonisation period and allows re-entry of patients carrying carbapenamase-producing bacteria into medical care facilities. *Int J Antimicrob Agents* 2019; **53**(4): 355-61.

199. Bar-Yoseph H, Carasso S, Shklar S, et al. Oral Capsulized Fecal Microbiota Transplantation for Eradication of Carbapenemase-producing Enterobacteriaceae Colonization With a Metagenomic Perspective. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2021; **73**(1): e166-e75.

200. Ghani R, Mullish BH, McDonald JAK, et al. Disease Prevention Not Decolonization: A Model for Fecal Microbiota Transplantation in Patients Colonized With Multidrug-resistant Organisms. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2021; **72**(8): 1444-7.

201. Biehl LM, Cruz Aguilar R, Farowski F, et al. Fecal microbiota transplantation in a kidney transplant recipient with recurrent urinary tract infection. *Infection* 2018; **46**(6): 871-4.

202. Wang T, Kraft CS, Woodworth MH, Dhere T, Eaton ME. Fecal Microbiota Transplant for Refractory Clostridium difficile Infection Interrupts 25-Year History of Recurrent Urinary Tract Infections. *Open forum infectious diseases* 2018; **5**(2): ofy016.

203. Hocquart M, Pham T, Kuete E, Tomei E, Lagier JC, Raoult D. Successful Fecal Microbiota Transplantation in a Patient Suffering From Irritable Bowel Syndrome and Recurrent Urinary Tract Infections. *Open forum infectious diseases* 2019; **6**(10): ofz398.

204. Grosen AK, Povlsen JV, Lemming LE, Jørgensen SMD, Dahlerup JF, Hvas CL. Faecal Microbiota Transplantation Eradicated Extended-Spectrum Beta-Lactamase-Producing Klebsiella pneumoniae from a Renal Transplant Recipient with Recurrent Urinary Tract Infections. *Case Rep Nephrol Dial* 2019; **9**(2): 102-7.

205. Aira A, Rubio E, Vergara Gómez A, et al. rUTI Resolution After FMT for Clostridioides difficile Infection: A Case Report. *Infect Dis Ther* 2020.

206. Jeney SES, Lane F, Oliver A, Whiteson K, Dutta S. Fecal Microbiota Transplantation for the Treatment of Refractory Recurrent Urinary Tract Infection. *Obstet Gynecol* 2020; **136**(4): 771-3.

207. Tariq R, Pardi DS, Tosh PK, Walker RC, Razonable RR, Khanna S. Fecal Microbiota Transplantation for Recurrent Clostridium difficile Infection Reduces Recurrent Urinary Tract Infection Frequency. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2017; **65**(10): 1745-7.

208. Ianiro G, Murri R, Sciumè GD, et al. Incidence of Bloodstream Infections, Length of Hospital Stay, and Survival in Patients With Recurrent Clostridioides difficile Infection Treated With Fecal Microbiota Transplantation or Antibiotics: A Prospective Cohort Study. *Annals of internal medicine* 2019; **171**(10): 695-702.

209. Gelb DJ, Oliver E, Gilman S. Diagnostic criteria for Parkinson disease. *Archives of neurology* 1999; **56**(1): 33-9.

210. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry* 1992; **55**(3): 181-4.

211. Chou KL. UpToDate: Clinical manifestations of Parkinson disease. 2022. Available from: https://www.uptodate.com/contents/clinical-manifestations-of-parkinson-disease?search=parkinson%20etiology&source=search_result&selectedTitle=4~150&usage_type=default&display_rank=4 (Accesed on the 16th of June 2022).

212. Williams-Gray CH, Mason SL, Evans JR, et al. The CamPaIGN study of Parkinson's disease: 10-year outlook in an incident population-based cohort. *J Neurol Neurosurg Psychiatry* 2013; **84**(11): 1258-64.

213. Macleod AD, Taylor KS, Counsell CE. Mortality in Parkinson's disease: a systematic review and meta-analysis. *Movement disorders : official journal of the Movement Disorder Society* 2014; **29**(13): 1615-22.

214. Pringsheim T, Jette N, Frolkis A, Steeves TD. The prevalence of Parkinson's disease: a systematic review and meta-analysis. *Movement disorders : official journal of the Movement Disorder Society* 2014; **29**(13): 1583-90.

215. Collaborators GN. Global, regional, and national burden of Parkinson's disease, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol* 2018; **17**(11): 939-53.

216. Ou Z, Pan J, Tang S, et al. Global Trends in the Incidence, Prevalence, and Years Lived With Disability of Parkinson's Disease in 204 Countries/Territories From 1990 to 2019. *Front Public Health* 2021; **9**: 776847.

217. Eimers M, van Erkelens J, van Tilburg C. ParkinsonNet in cijfers. Trends in paramedische zorg tussen 2010-2020 (2021). Available from: https://www.parkinsonnet.nl/app/uploads/2021/09/ParkinsonNet-in-cijfers-paramedische-zorg-2010-2020.pdf (Accessed on the 7th of Januari 2023).

218. Jankovic, J. UptoDate: Etiology and pathogenesis of Parkinson disease. 2021. Available from: https://www.uptodate.com/contents/etiology-and-pathogenesis-of-parkinson-disease?search=parkinson%20etiology&source=search_result&selectedTitle=1~150&usage_type=default&display_rank=1 (Accessed on the 16th of June 2022).

219. Pakkenberg B, Moller A, Gundersen HJ, Mouritzen Dam A, Pakkenberg H. The absolute number of nerve cells in substantia nigra in normal subjects and in patients with Parkinson's disease estimated with an unbiased stereological method. *J Neurol Neurosurg Psychiatry* 1991; **54**(1): 30-3.

220. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. *Nature* 1997; **388**(6645): 839-40.

221. Liu L, Huh JR, Shah K. Microbiota and the gut-brain-axis: Implications for new therapeutic design in the CNS. *EBioMedicine* 2022; **77**: 103908.

222. Postuma RB, Aarsland D, Barone P, et al. Identifying prodromal Parkinson's disease: pre-motor disorders in Parkinson's disease. *Movement disorders : official journal of the Movement Disorder Society* 2012; **27**(5): 617-26.

223. Poewe W. Non-motor symptoms in Parkinson's disease. *European journal of neurology* 2008; **15 Suppl 1**: 14-20.

224. Braak H, Rub U, Gai WP, Del Tredici K. Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. *Journal of neural transmission (Vienna, Austria : 1996)* 2003; **110**(5): 517-36.

225. Shannon KM, Keshavarzian A, Dodiya HB, Jakate S, Kordower JH. Is alpha-synuclein in the colon a biomarker for premotor Parkinson's disease? Evidence from 3 cases. *Movement disorders : official journal of the Movement Disorder Society* 2012; **27**(6): 716-9.

226. Hallett PJ, McLean JR, Kartunen A, Langston JW, Isacson O. alpha-Synuclein overexpressing transgenic mice show internal organ pathology and autonomic deficits. *Neurobiology of disease* 2012; **47**(2): 258-67.

227. Wang L, Magen I, Yuan PQ, et al. Mice overexpressing wild-type human alpha-synuclein display alterations in colonic myenteric ganglia and defecation. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* 2012; **24**(9): e425-36.

228. Kuo YM, Li Z, Jiao Y, et al. Extensive enteric nervous system abnormalities in mice transgenic for artificial chromosomes containing Parkinson disease-associated alpha-synuclein gene mutations precede central nervous system changes. *Human molecular genetics* 2010; **19**(9): 1633-50.

229. Stokholm MG, Danielsen EH, Hamilton-Dutoit SJ, Borghammer P. Pathological alpha-synuclein in gastrointestinal tissues from prodromal Parkinson disease patients. *Annals of neurology* 2016; **79**(6): 940-9.

230. Liautard JP. Are prions misfolded molecular chaperones? *FEBS letters* 1991; **294**(3): 155-7.

231. Desplats P, Lee HJ, Bae EJ, et al. Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein. *Proc Natl Acad Sci U S A* 2009; **106**(31): 13010-5.

232. Holmqvist S, Chutna O, Bousset L, et al. Direct evidence of Parkinson pathology spread from the gastrointestinal tract to the brain in rats. *Acta neuropathologica* 2014; **128**(6): 805-20.

233. Ulusoy A, Rusconi R, Perez-Revuelta BI, et al. Caudo-rostral brain spreading of alpha-synuclein through vagal connections. *EMBO molecular medicine* 2013; **5**(7): 1119-27.

234. Pan-Montojo F, Anichtchik O, Dening Y, et al. Progression of Parkinson's disease pathology is reproduced by intragastric administration of rotenone in mice. *PloS one* 2010; **5**(1): e8762.

235. Shults CW. Lewy bodies. *Proc Natl Acad Sci U S A* 2006; **103**(6): 1661-8.

236. Keshavarzian A, Green SJ, Engen PA, et al. Colonic bacterial composition in Parkinson's disease. *Movement disorders : official journal of the Movement Disorder Society* 2015; **30**(10): 1351-60.

237. Forsyth CB, Shannon KM, Kordower JH, et al. Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. *PloS one* 2011; **6**(12): e28032.

238. Devos D, Lebouvier T, Lardeux B, et al. Colonic inflammation in Parkinson's disease. *Neurobiology of disease* 2013; **50**: 42-8.

239. Sampson TR, Debelius JW, Thron T, et al. Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. *Cell* 2016; **167**(6): 1469-80.e12.

240. Sun MF, Zhu YL, Zhou ZL, et al. Neuroprotective effects of fecal microbiota transplantation on MPTP-induced Parkinson's disease mice: Gut microbiota, glial reaction and TLR4/TNF-alpha signaling pathway. *Brain, behavior, and immunity* 2018; **70**: 48-60.

241. Zhou ZL, Jia XB, Sun MF, et al. Neuroprotection of Fasting Mimicking Diet on MPTP-Induced Parkinson's Disease Mice via Gut Microbiota and Metabolites. *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics* 2019; **16**(3): 741-60.

242. Unger MM, Spiegel J, Dillmann KU, et al. Short chain fatty acids and gut microbiota differ between patients with Parkinson's disease and age-matched controls. *Parkinsonism & related disorders* 2016; **32**: 66-72.

243. Hasegawa S, Goto S, Tsuji H, et al. Intestinal Dysbiosis and Lowered Serum Lipopolysaccharide-Binding Protein in Parkinson's Disease. *PloS one* 2015; **10**(11): e0142164.

244. Scheperjans F, Aho V, Pereira PA, et al. Gut microbiota are related to Parkinson's disease and clinical phenotype. *Movement disorders : official journal of the Movement Disorder Society* 2015; **30**(3): 350-8.

245. Petrov VA, Saltykova IV, Zhukova IA, et al. Analysis of Gut Microbiota in Patients with Parkinson's Disease. *Bulletin of experimental biology and medicine* 2017; **162**(6): 734-7.

246. Hill-Burns EM, Debelius JW, Morton JT, et al. Parkinson's disease and Parkinson's disease medications have distinct signatures of the gut microbiome. *Movement disorders : official journal of the Movement Disorder Society* 2017; **32**(5): 739-49.

247. Hopfner F, Kunstner A, Muller SH, et al. Gut microbiota in Parkinson disease in a northern German cohort. *Brain research* 2017; **1667**: 41-5.

248. Li W, Wu X, Hu X, et al. Structural changes of gut microbiota in Parkinson's disease and its correlation with clinical features. *Science China Life sciences* 2017; **60**(11): 1223-33.

249. Romano S, Savva GM, Bedarf JR, Charles IG, Hildebrand F, Narbad A. Meta-analysis of the Parkinson's disease gut microbiome suggests alterations linked to intestinal inflammation. *NPJ Parkinsons Dis* 2021; **7**(1): 27.

250. Bedarf JR, Hildebrand F, Coelho LP, et al. Functional implications of microbial and viral gut metagenome changes in early stage L-DOPA-naive Parkinson's disease patients. *Genome medicine* 2017; **9**(1): 39.

251. Lin CH, Chen CC, Chiang HL, et al. Altered gut microbiota and inflammatory cytokine responses in patients with Parkinson's disease. *Journal of neuroinflammation* 2019; **16**(1): 129.

252. Wallen ZD, Demirkan A, Twa G, et al. Metagenomics of Parkinson's disease implicates the gut microbiome in multiple disease mechanisms. *Nature communications* 2022; **13**(1): 6958.

253. Talavera Andújar B, Aurich D, Aho VTE, et al. Studying the Parkinson's disease metabolome and exposome in biological samples through different analytical and cheminformatics approaches: a pilot study. *Anal Bioanal Chem* 2022; **414**(25): 7399-419.

254. Schwiertz A, Spiegel J, Dillmann U, et al. Fecal markers of intestinal inflammation and intestinal permeability are elevated in Parkinson's disease. *Parkinsonism & related disorders* 2018; **50**: 104-7.

255. Varesi A, Campagnoli LIM, Fahmideh F, et al. The Interplay between Gut Microbiota and Parkinson's Disease: Implications on Diagnosis and Treatment. *International journal of molecular sciences* 2022; **23**(20).

256. Goetz CG, Tilley BC, Shaftman SR, et al. Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results. *Movement disorders : official journal of the Movement Disorder Society* 2008; **23**(15): 2129-70.

257. Chen SJ, Chen CC, Liao HY, et al. Association of Fecal and Plasma Levels of Short-Chain Fatty Acids With Gut Microbiota and Clinical Severity in Patients With Parkinson Disease. *Neurology* 2022; **98**(8): e848-e58.

258. Mao L, Zhang Y, Tian J, et al. Cross-Sectional Study on the Gut Microbiome of Parkinson's Disease Patients in Central China. *Frontiers in microbiology* 2021; **12**: 728479.

259. Barichella M, Severgnini M, Cilia R, et al. Unraveling gut microbiota in Parkinson's disease and atypical parkinsonism. *Movement disorders : official journal of the Movement Disorder Society* 2019; **34**(3): 396-405.

260. Pietrucci D, Cerroni R, Unida V, et al. Dysbiosis of gut microbiota in a selected population of Parkinson's patients. *Parkinsonism & related disorders* 2019; **65**: 124-30.

261. Li Z, Lu G, Luo E, et al. Oral, Nasal, and Gut Microbiota in Parkinson's Disease. *Neuroscience* 2022; **480**: 65-78.

262. Sampson TR, Challis C, Jain N, et al. A gut bacterial amyloid promotes α-synuclein aggregation and motor impairment in mice. *Elife* 2020; **9**.

263. Chen SG, Stribinskis V, Rane MJ, et al. Exposure to the Functional Bacterial Amyloid Protein Curli Enhances Alpha-Synuclein Aggregation in Aged Fischer 344 Rats and Caenorhabditis elegans. *Scientific reports* 2016; **6**: 34477.

264. Wang C, Lau CY, Ma F, Zheng C. Genome-wide screen identifies curli amyloid fibril as a bacterial component promoting host neurodegeneration. *Proc Natl Acad Sci U S A* 2021; **118**(34).

265. Murros KE. Hydrogen Sulfide Produced by Gut Bacteria May Induce Parkinson's Disease. *Cells* 2022; **11**(6).

266. Murros KE, Huynh VA, Takala TM, Saris PEJ. Desulfovibrio Bacteria Are Associated With Parkinson's Disease. *Frontiers in cellular and infection microbiology* 2021; **11**: 652617.

267. Wilmes P, Trezzi J, Aho V, et al. An archaeal compound as a driver of Parkinson's disease pathogenesis, 26 July 2022, PREPRINT (Version 1) available at Research Square [https://doi.org/10.21203/rs.3.rs-1827631/v1].

268. Yamasaki TR, Ono K, Ho L, Pasinetti GM. Gut Microbiome-Modified Polyphenolic Compounds Inhibit α-Synuclein Seeding and Spreading in α-Synucleinopathies. *Front Neurosci* 2020; **14**: 398.

269. van Kessel SP, Frye AK, El-Gendy AO, et al. Gut bacterial tyrosine decarboxylases restrict levels of levodopa in the treatment of Parkinson's disease. *Nature communications* 2019; **10**(1): 310.

270. Maini Rekdal V, Bess EN, Bisanz JE, Turnbaugh PJ, Balskus EP. Discovery and inhibition of an interspecies gut bacterial pathway for Levodopa metabolism. *Science (New York, NY)* 2019; **364**(6445).

271. Lubomski M, Tan AH, Lim SY, Holmes AJ, Davis RL, Sue CM. Parkinson's disease and the gastrointestinal microbiome. Journal of neurology 2020; 267(9): 2507-23. https://doi.org/10.1007/s00415-019-09320-1.

272. Tamtaji OR, Taghizadeh M, Daneshvar Kakhaki R, et al. Clinical and metabolic response to probiotic administration in people with Parkinson's disease: A randomized, double-blind, placebo-controlled trial. *Clinical nutrition (Edinburgh, Scotland)* 2019; **38**(3): 1031-5.

273. Sun H, Zhao F, Liu Y, et al. Probiotics synergized with conventional regimen in managing Parkinson's disease. *NPJ Parkinsons Dis* 2022; **8**(1): 62.

274. Lu CS, Chang HC, Weng YH, Chen CC, Kuo YS, Tsai YC. The Add-On Effect of Lactobacillus plantarum PS128 in Patients With Parkinson's Disease: A Pilot Study. *Front Nutr* 2021; **8**: 650053.

275. Hong CT, Chen JH, Huang TW. Probiotics treatment for Parkinson disease: a systematic review and meta-analysis of clinical trials. *Aging* 2022; **14**(17): 7014-25.

276. Yin S, Zhu F. Probiotics for constipation in Parkinson's: A systematic review and meta-analysis of randomized controlled trials. *Frontiers in cellular and infection microbiology* 2022; **12**: 1038928.

277. Becker A, Schmartz GP, Gröger L, et al. Effects of Resistant Starch on Symptoms, Fecal Markers, and Gut Microbiota in Parkinson's Disease - The RESISTA-PD Trial. *Genomics Proteomics Bioinformatics* 2022; **20**(2): 274-87.

278. Cantu-Jungles TM, Rasmussen HE, Hamaker BR. Potential of Prebiotic Butyrogenic Fibers in Parkinson's Disease. *Frontiers in neurology* 2019; **10**: 663.

279. Paiva I, Pinho R, Pavlou MA, et al. Sodium butyrate rescues dopaminergic cells from alpha-synuclein-induced transcriptional deregulation and DNA damage. *Human molecular genetics* 2017; **26**(12): 2231-46.

280. St Laurent R, O'Brien LM, Ahmad ST. Sodium butyrate improves locomotor impairment and early mortality in a rotenone-induced Drosophila model of Parkinson's disease. *Neuroscience* 2013; **246**: 382-90.

281. Zhou W, Bercury K, Cummiskey J, Luong N, Lebin J, Freed CR. Phenylbutyrate up-regulates the DJ-1 protein and protects neurons in cell culture and in animal models of Parkinson disease. *The Journal of biological chemistry* 2011; **286**(17): 14941-51.

282. Dong XL, Wang X, Liu F, et al. Polymannuronic acid prevents dopaminergic neuronal loss via brain-gut-microbiota axis in Parkinson's disease model. *Int J Biol Macromol* 2020; **164**: 994-1005.

283. Liu X, Du ZR, Wang X, et al. Polymannuronic acid prebiotic plus Lacticaseibacillus rhamnosus GG probiotic as a novel synbiotic promoted their separate neuroprotection against Parkinson's disease. *Food Res Int* 2022; **155**: 111067.

284. Barichella M, Pacchetti C, Bolliri C, et al. Probiotics and prebiotic fiber for constipation associated with Parkinson disease: An RCT. *Neurology* 2016; **87**(12): 1274-80.

285. Ahmed S, Busetti A, Fotiadou P, et al. In vitro Characterization of Gut Microbiota-Derived Bacterial Strains With Neuroprotective Properties. *Front Cell Neurosci* 2019; **13**: 402.

286. Fang X, Zhou X, Miao Y, Han Y, Wei J, Chen T. Therapeutic effect of GLP-1 engineered strain on mice model of Alzheimer's disease and Parkinson's disease. *AMB Express* 2020; **10**(1): 80.

287. Lorente-Picón M, Laguna A. New Avenues for Parkinson's Disease Therapeutics: Disease-Modifying Strategies Based on the Gut Microbiota. *Biomolecules* 2021; **11**(3).

288. Lin CH, Lai HC, Wu MS. Gut-oriented disease modifying therapy for Parkinson's disease. *J Formos Med Assoc* 2023; **122**(1): 9-18.

289. Knight E, Geetha T, Burnett D, Babu JR. The Role of Diet and Dietary Patterns in Parkinson's Disease. *Nutrients* 2022; **14**(21).

290. Figueiredo M. Phase 1 Trial Set to Test First Live Biotherapeutics in Parkinson's (2022). Available from: https://parkinsonsnewstoday.com/news/phase-1-trial-will-evaluate-first-live-biotherapeutics-parkinsons-patients/ (Accessed on the 7th of January 2023).

291. Sheng S, Zhao S, Zhang F. Insights into the roles of bacterial infection and antibiotics in Parkinson's disease. *Frontiers in cellular and infection microbiology* 2022; **12**: 939085.

292. Huang H, Xu H, Luo Q, et al. Fecal microbiota transplantation to treat Parkinson's disease with constipation: A case report. *Medicine* 2019; **98**(26): e16163.

293. Xue LJ, Yang XZ, Tong Q, et al. Fecal microbiota transplantation therapy for Parkinson's disease: A preliminary study. *Medicine* 2020; **99**(35): e22035.

294. Segal A, Zlotnik Y, Moyal-Atias K, Abuhasira R, Ifergane G. Fecal microbiota transplant as a potential treatment for Parkinson's disease - A case series. *Clin Neurol Neurosurg* 2021; **207**: 106791.

295. Kuai XY, Yao XH, Xu LJ, et al. Evaluation of fecal microbiota transplantation in Parkinson's disease patients with constipation. *Microb Cell Fact* 2021; **20**(1): 98.