C. difficile - An intestinal germ with pathogenic potential

FAQ of BfR of 20. July 2023

Clostridioides (*C.*) *difficile* is a bacterium that can colonise the human intestine without causing symptoms. However, it can also cause infections associated with diseases of the gastrointestinal tract, if the bacterial community in the human intestine has been disturbed. Therefore, most *C. difficile* infections occur in connection with antibiotic therapy and/or hospitalisation. In recent years, *C. difficile* infections, which occurred independently of these known risk factors, have been increasingly reported in Germany. Possible causes include contaminated food and contact with livestock.

C. difficile occurs everywhere in the environment and in the gastrointestinal tract of humans and animals. Therefore, in principle, several transmission routes are conceivable. Here, the German Federal Institute for Risk Assessment (BfR) has compiled questions and answers on *C. difficile* and its significance along the food chain. Further information on medical questions is available from the Robert Koch Institute (RKI).

What is *C. difficile*?

Clostridioides difficile - formerly *Clostridium difficile* - is a widespread Gram-positive bacterium. It multiplies only in the absence of oxygen and is capable of forming resistant developmental stages called spores. *C. difficile* is found both in the environment, e.g. in soils, and in the gastrointestinal tract of healthy humans and animals. According to studies, *C. difficile* can be detected in the stool of about 5 % of healthy adults.

Under certain circumstances, the bacterium is capable of producing toxins that can cause diarrhoea of varying severity. According to US studies, *C. difficile* is responsible for 15-20 % of antibiotic-associated diarrhoea. Infections are particularly common in hospital patients. Different *C. difficile* strains are characterised, among other things, by their ability to produce toxins.

C. difficile belongs to the Clostridia class of bacteria. This group includes several representatives that can cause illness in humans or animals through the production of toxins. These include, among others, the tetanus pathogen *Clostridium tetani* and *Clostridium botulinum*, the causative agent of botulism.

What is the difference between *C. difficile* spores and *C. difficile* cells?

Cells are the metabolically active forms of *C. difficile* – the cells are actively growing and multiplying. Only in this state, the production of toxins is possible. As a spore, the bacterium is in an inert state. The spore is a very resistant state that the bacterium assumes when the environmental conditions are unfavourable for growth and multiplication. Unlike cells, spores are resistant to drought, heat and some disinfectants, among other things. Only when environmental conditions are favourable, the spore germinates into a cell capable of multiplying.

How do the types of *C. difficile* differ?

The ability to produce toxins is one of the essential distinguishing features of the different *C. difficile* strains. The pathogenic (disease-causing) strains can produce up to three toxins: Enterotoxin A and/or Cytotoxin B, and in some strains additionally the so-called binary toxin (CDT). Strains that do not produce toxins are considered apathogenic (non disease-causing).

Furthermore, *C. difficile* strains are classified according to so-called ribotypes (RT) (see following question). Some ribotypes are more strongly associated with human infections than others. For example, strains of RT027 are considered particularly virulent and cause severe courses of the disease more often.

Some laboratories use multi-locus-sequence typing instead of ribotyping (see following question). Often, the sequence types determined in this way can correlate with certain ribotypes, but synonymous use is not possible.

What is the difference between ribotyping and multi-locus-sequence typing?

Ribotyping and multi-locus-sequence typing are methods for classifying bacteria.

In ribotyping, certain DNA regions of the bacteria – the so-called ribosomal DNA – are cut into fragments of different sizes by specific enzymes. The length of these fragments and the fragment patterns are then used to determine the so-called ribotype.

In contrast, multi-locus sequence typing looks at the different variants of a series of genes. Based on these variants, the so-called sequence type can be determined.

How do the toxins produced by *C. difficile* work?

All *C. difficile* toxins – enterotoxin A, cytotoxin B and the binary toxin – destroy the cells of the intestinal mucosa. This leads to an increased release of fluids into the intestine, which are excreted as diarrhoea.

Which diseases are caused by *C. difficile*?

C. difficile mainly causes diarrhoea. It is usually watery diarrhoea accompanied by fever and abdominal pain. In severe cases, bloody diarrhoea can also occur. Possible complications include intestinal obstruction, acute dilatation of the colon (toxic megacolon) or rupture of the intestinal wall and even blood poisoning (septicaemia). According to US studies, about 15-20% of diarrhoea associated with antibiotic administration is caused by *C. difficile*. Recurrent episodes of diarrhoea (recurrent cases) are also problematic, especially when they occur in already weakened individuals.

How often do *C. difficile* infections occur in humans in Germany?

In Germany, the number of reported cases has been declining for several years. However, according to the Infection Protection Act (IfSG§6), only illness and death related to *C. difficile* infections with a clinically severe course must be reported nationwide. The Robert Koch Institute (RKI) has recorded 1,301 cases with severe courses in 2022. In Saxony, additionally, all intestinal infections involving *C. difficile* are notifiable. Here, more than 2,700 *C. difficile* infections were reported in 2022. In the same period, 123 cases with clinically severe courses were recorded in Saxony. These figures illustrate that *C. difficile* infections in humans are relatively common in Germany, but are usually mild.

Is antibiotic resistance a problem in connection with *C. difficile*?

A healthy bacterial community in the intestine consists of various bacteria, some of which produce inhibitors that prevent the colonisation with *C. difficile*. When antibiotics are administered because of an infection with another pathogen, these harmless intestinal bacteria can be killed, as an undesirable side effect. The resulting lack of inhibitors and the now available

nutrients allow *C. difficile* to multiply and spread in the intestine. This is because *C. difficile* itself is resistant to a large number of antibiotics and therefore survives the antibiotic treatment without damage. However, resistances to antibiotics used as therapy for *C. difficile* infections are rare.

Who is particularly at risk?

An older age (> 65 years), a previous antibiotic therapy as well as hospitalization, stay in a nursing home or a similar institution are the most important risk factors for *C. difficile* infections. In addition, pre-existing illnesses in which the body's own defenses are reduced and the intake of certain medications e.g. against heartburn (antacids) can favour a *C. difficile* infection. Overall, the infections are often associated with a bacterial community in the intestine that has been disturbed by illness or medication. In recent years, however, younger people without known risk factors have been increasingly affected. Possible causes include contaminated food and contact with livestock.

How can humans become infected with *C. difficile*?

Humans can become infected via oral ingestion of spores or cells. The spores germinate in the intestine to form vegetative cells that are capable of producing toxins. It usually takes only a few days for the first symptoms to appear. With the stool of infected or colonised humans and animals, cells and spores enter the environment. Poor hand hygiene can lead to smear infections.

A healthy bacterial community in the intestine can prevent *C. difficile* colonisation or infection. Therefore, a disturbed bacterial community in the intestine, e.g. due to pre-existing illnesses or antibiotic therapy often precedes the development of a *C. difficile* infection.

What is the difference between an infection and a colonisation with *C. difficile*?

When colonised, *C. difficile* occurs in the gastrointestinal tract of healthy people without causing symptoms. Under certain circumstances (e.g. antibiotic administration), colonisation can turn into an infection with clinical symptoms. It is discussed whether colonisation with *C. difficile* strains that do not produce toxins (apathogenic strains) protects against infection with disease-causing (pathogenic) strains. However, a transfer of the toxin-producing ability from pathogenic to apathogenic strains is conceivable. Because the ability to produce toxins can also be located on so-called mobile genetic elements that can be exchanged between different bacteria. In such a case, the originally protective, apathogenic strains could themselves contribute to a *C. difficile* infection.

How common is *C. difficile* colonisation in humans?

The probability of colonisation depends, among other things, on age. According to international studies, between 30 and 70 % of infants carry the pathogen in their intestines. In contrast, only up to 5 % of adults are colonised, with the frequency increasing again with age. During hospitalisation, the frequency of colonised patients increases with the length of the stay. About 5-30 % of the residents of a care facility carry the pathogen.

Does *C. difficile* also occur in livestock?

C. difficile is found in the gastrointestinal tract of livestock and can enter the environment via faeces. A higher colonisation rate is found in young animals than in adult animals. Infections

with *C. difficile* can occur in livestock of all ages. For example, pigs, cattle and poultry are affected.

Can *C. difficile* be transmitted between humans and animals?

There are so-called zoonotic ribotypes (e.g. RT078) for which transmission between animals and humans is likely, as they are found in both animals and humans. Genetic studies of strains of these ribotypes from animals partly show a high degree of similarity to strains from diseased or colonised humans, which also suggests transmission. However, the evidence for this is still lacking.

This may be due to the fact that colonisation can remain undetected for a long time, as the presence of *C. difficile* is only examined when corresponding symptoms are present. Diseases can occur when the healthy bacterial community of the intestine is disturbed and *C. difficile* multiplies excessively. Due to the time interval, the colonisation can then no longer be traced back to a specific event.

Another possible explanation for the high genetic similarity between strains from livestock and humans is the transmission of the pathogen from a common other source, for example from the soil or from plants.

Does *C. difficile* also occur in food?

C. difficile apparently occurs worldwide in various foods of plant or animal origin with different frequencies. Investigations by the BfR showed that the pathogen is frequently detectable on fresh chicken meat products with skin and on potatoes. In an investigation of salad samples from supermarkets in the Berlin-Brandenburg area, however, *C. difficile* was only found very rarely.

How does *C. difficile* get into food?

C. difficile is found in the gastrointestinal tract of humans and animals and is also widespread in the environment, e.g. in soils. In foods of animal origin, *C. difficile* can enter the food directly through faecal contamination during production or subsequently through contamination during further processing. In plant foods, *C. difficile* can be transferred to the food via the soil in which the food is grown, via contaminated natural fertilisers or via humans during further processing.

Can humans become infected with *C. difficile* through consumption of food?

Due to the similarity of *C. difficile* strains found on food and in (healthy and diseased) humans, transmission via food seems possible in principle. However, this has not yet been proven. It is conceivable that colonisation with the bacterium takes place via contaminated food and remains symptom-free until a disturbance of the intestinal bacterial community occurs. The following possible disease is difficult to attribute to a cause due to the time interval between colonisation and infection.

How can consumers protect themselves from *C. difficile* colonisation?

It is not possible for consumers to tell, if food is contaminated with *C. difficile*. The pathogen can only be detected by extensive laboratory tests.

Good hand hygiene is important to protect against *C. difficile* colonisation. However, disinfecting the hands can only kill the *C. difficile* cells and not the spores, as these are resistant to disinfectants (e.g. alcohol). Therefore, hand washing with soap and water is recommended to reduce spore contamination of the hands.

To minimise the risk of food transmission, general kitchen hygiene rules should be followed, as found in the leaflet "Protection against foodborne infections in private households" (https://www.bfr.bund.de/cm/364/protection-against-foodborne-infections.pdf).

In which areas does the BfR conduct research on *C. difficile*?

The BfR's laboratory for spore formers deals with *C. difficile* within the framework of national and international research projects. Among other things, methods for detecting spores, cells and toxin genes as well as for antibiotic susceptibility testing are developed and optimised. Furthermore, the BfR, also together with the federal states, is investigating the occurrence of *C. difficile* in various foods of animal and plant origin. The strains found here are characterised with regard to ribotype, toxin-producing ability and existing resistances and the relationship to human strains.

Further information on the BfR website on the topic of clostridia:

More information on Clostridia
https://www.bfr.bund.de/en/clostridien-54349.html

Rare but avoidable: Questions and answers on botulism
https://www.bfr.bund.de/cm/349/rare-but-avoidable-questions-and-answers-about-botulism.44719717.pdf

About the BfR

The German Federal Institute for Risk Assessment (BfR) is a scientifically independent institution within the portfolio of the Federal Ministry of Food and Agriculture (BMEL) in Germany. The BfR advises the Federal Government and the States (‘Laender’) on questions of food, chemical and product safety. The BfR conducts its own research on topics that are closely linked to its assessment tasks.