

## Prevalence of *Clostridioides difficile* in strawberries from Germany

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### INTRODUCTION

*Clostridioides difficile* (formerly *Clostridium*) is a spore-forming anaerobe and a potential intestinal pathogen for a wide range of mammals, including humans. People with disturbed intestinal microbiota due to immunosuppression or antimicrobial treatment are particularly at risk. Besides the well-known nosocomial infections, community-associated *C. difficile* infections (CDI) are on the rise. In these cases, younger people who have not undergone antimicrobial therapy or been hospitalised are also affected, raising the question of the source of infection. Since high genetic relatedness was identified between isolates from human cases and those collected from food, contaminated food might be a source of community-associated CDI. In the past, *C. difficile* was frequently isolated from different vegetables, especially those growing with close contact to soil. We wanted to focus our research on strawberries, which are usually cultivated on straw above soil. We were especially interested in berries from self-pick-fields, since these often lead to the consumption of unwashed fruit, which could potentially pose the risk of *C. difficile* colonisation or infection.

### METHODOLOGY

In the summer of 2021, strawberry samples were collected at 25 locations (self-pick-fields, on-field selling booths, supermarkets (including organic producers), outdoor markets and a garden). For every location, two pooled samples of 100 g were collected, resulting in 50 samples in total. Berries were stored for a maximum of three days in the fridge prior to testing. The surface of the strawberries was scrubbed with a sponge, and they were then incubated for seven days under strictly anaerobic conditions at 37°C in a Stomacher bag containing 100 ml of brain heart infusion supplemented with moxalactam/norfloxacin (Oxoid) and 0.1 % sodium taurocholate. After incubation, the enrichment culture with and without ethanol shock was streaked onto ChromID *C. difficile* agar plates (bioMérieux). After incubation for two days at 37°C under strictly anaerobic conditions, presumptive *C. difficile* colonies were transferred to Columbia Blood Agar and submitted to species identification via MALDI-TOF. Further characterisation will include PCR-ribotyping and toxin gene detection.

## RESULTS

29 samples from 20 locations showed growth (sometimes black streaks across the whole plate, without distinctive colonies) on the selective plates. Of these, 20 could be cultured on blood agar and were subject to MALDI-TOF analysis for species identification. In seven samples, no identification was possible via MALDI-TOF. The other 13 samples harboured a variety of anaerobes. Regarding the Clostridiaceae family, they contained *Clostridioides difficile* (n = 1), *Clostridium celerecrescens* (n = 3) and *Clostridium sphenoides* (n = 1). Other identified species were *Lactobacillus* spp. (n = 5), *Paenibacillus lactis* (n = 3), *Bacteroides xyloxyticus* (n = 2) and *Terrisporobacter glycolicus* (n = 1). Two samples from different locations simultaneously harboured *L. brevis* and *P. lactis*. In both samples, *L. brevis* was identified without ethanol shock and *P. lactis* was identified with preceding ethanol-shock. The sample containing *C. sphenoides* also included *C. celerecrescens*. *C. difficile* was found on strawberries from a self-pick-field in the federal state of Brandenburg. The other *Clostridium* species were found on strawberries from an outdoor market, a second self-pick-field and a selling booth directly by a third field.

## DISCUSSION

The goal of our study was to identify the prevalence of *C. difficile* in strawberries. Due to the short strawberry season, the sampling was limited regarding region and number of samples, which in effect limits the scope of our study. Only one of fifty samples (2 %) was positive for *C. difficile*, hinting towards a possible transmission route. CDI is a multifactorial disease and other factors, such as disturbed intestinal microbiota, need to coincide for symptoms to develop. However, on self-pick-fields, fruit is often consumed unwashed, so the presence of *C. difficile* on strawberries from a self-pick-field might lead to the colonisation of a host, which could result in CDI, when other factors apply. The identification of the PCR-ribotype and the investigation of the genetic relatedness of the strawberry isolates and isolates from human infection will help to estimate the risk of colonisation or infection via this route. The other isolated clostridial species are mostly irrelevant regarding foodborne disease. Only *C. sphenoides* was identified as the cause of severe diarrhoea in a single case report from 1980. Overall, the prevalence of *C. difficile* in strawberries seems to be low.