

## Note

## Low prevalence of *Clostridium septicum* fecal carriage in an adult population



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### ABSTRACT

*Clostridium septicum* is an uncommon cause of severe infection. Real-time PCR against the *C. septicum*-specific alpha-toxin gene (*csa*) was used to estimate the prevalence of this microbe in human stool from 161 asymptomatic community-dwelling adults and 192 hospitalized patients with diarrhea. All samples were negative, suggesting a low prevalence.

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*Vibrio septique*, later known as *Clostridium septicum*, was one of the first pathogenic anaerobes to be identified [1]. It is a toxigenic, anaerobic, Gram positive, spore-forming bacillus that is an uncommon cause of human infections [2–4]. Severe infections can be accompanied by refractory toxic shock, and there is a remarkable association of such infections with the presence of malignancy, either solid tumors or hematologic [5–7]. The severe clinical syndrome that accompanies invasive *C. septicum* infections relates to its expression of a pore forming alpha toxin encoded by the *csa* gene, which appears to be carried by all strains of this bacterium [8–10]. When they occur, *C. septicum* infections result primarily from hematogenous spread, involving either primary bacteremia alone, or appearing as spontaneous soft tissue infections (or both) [2]. It is held that the gastrointestinal tract is a common point of access for *C. septicum* bacteremia, which may explain its close association with gastrointestinal tumors such as colon cancer [11–14]. Despite antibiotics and supportive care, disseminated

*C. septicum* infections remain dangerous. Unfortunately, there are no effective preventive measures against this rare outcome, in part because there is not a clear understanding of who is at the highest risk for developing fulminant infection.

Because colonization or transient carriage of *C. septicum* within the gastrointestinal tract is believed to be an important predisposing factor to infection it is important to better understand risk factors for such carriage. In this light, we tested the sensitivity of a *C. septicum*-specific real-time PCR approach in screening the prevalence of this organism in human stool specimens. A successful detection tool would help generate data that might be helpful for identifying patients at highest risk for infection. Although, *C. septicum* colonization occurs in humans [15,16], its frequency and stability is unclear.

Because the *csa* gene appears to be ubiquitous and unique among *C. septicum* strains, and there is a highly specific and sensitive real-time PCR assay for its detection [10], we applied this approach to screen for the presence of *C. septicum* in 192 hospitalized patients with diarrhea (who tested negative for *Clostridium difficile*) and 161 outpatients without symptoms of diarrhea.

This study was conducted as part of a larger study about *C. difficile* infection. Following Institutional Review Board approval,

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fecal specimens were collected from 161 outpatient adults without symptoms of gastroenteritis (age > 18 years) and 192 hospitalized patients with symptoms suggestive of *C. difficile* but tested negative for this pathogen in the University of Michigan Health System Clinical Microbiology Laboratory. The absence of *C. difficile* was verified by culture. Two reference *C. septicum* bacterial strains used in this study (CS0712 and CS120612) were de-identified, historical isolates collected from clinical specimens in the University of Michigan Health System Clinical Microbiology Laboratory and a third strain was Pasteur III (NCTC 547), obtained from the American Type Culture Collection (ATCC, Manassas, VA).

To establish a molecular approach for *C. septicum* detection, we determined the sensitivity of real-time PCR for detecting the *csa* gene of *C. septicum* using DNA prepared from reference strains. This gene is believed to be highly conserved in *C. septicum* and *csa*-negative strains have not been reported to our knowledge [17]. Each of three reference *C. septicum* strains was grown overnight, anaerobically in Reinforced Clostridial Media (RCM, Difco™; Beckton and Dickinson, Sparks, MD) broth and then subjected to DNA extraction using an Easy DNA kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The DNA samples (172 ng/μl) were serially 10-fold diluted from 10<sup>-1</sup> to 10<sup>-8</sup> and *csa* real-time PCR was run on a LightCycler 96 (Roche, Mannheim, Germany), as described in Ref. [17]. The highly specific *C. septicum* alpha-toxin (*csa*) gene primers used were those published by Neumann et al. [10]: Forward: 5'-TAGGATTGGATGGTGGGTG-GAA-3', Reverse: 5'-TGCACGATACCCACTTGCATAAGG-3'. Using these primers, *C. septicum* DNA was detected at a dilution as low as a 10<sup>-7</sup> (Fig. 1), corresponding to a concentration of 17.2 fg/μl. Cycle threshold (Ct) values close to 36 or higher, as observed for reactions using 10<sup>-8</sup> dilutions, were indistinguishable from values obtained from the negative control, and likely corresponded to undetectable levels of *C. septicum* DNA. Remarkably similar values were obtained for all three reference strains.

We next addressed the sensitivity of this PCR approach to detect DNA from bacteria spiked into human stool, using a single reference strain of *C. septicum*. On two separate occasions, an overnight broth culture of strain CS120612 culture was used to prepare a series of 10-fold dilutions, from undiluted to 10<sup>-6</sup> dilution. Each of these dilutions was then spiked into *C. difficile*- and *C. septicum*-negative human stool in bead beat tubes (MOBIO, Carlsbad, CA). These samples were then subjected to DNA isolation and real-time PCR.

Exact colony counts (CFU/ml) were determined for each of these broth dilutions based on overnight growth on solid medium. Stool DNA was isolated using MagNa Pure LC (Roche, Mannheim, Germany), and DNA was serially diluted, 10<sup>-1</sup> to 10<sup>-6</sup>. The real-time PCR was conducted as described above. Each PCR reaction was conducted in duplicate. The Ct values from stool spiked with a 10<sup>-6</sup> dilution of *C. septicum* were undefined because *C. septicum* was not present, or its concentration level was too low to be detected by the PCR assay. Based upon original concentrations of the CS120612 *C. septicum* culture used to spike the stool in experiment 1 of 6.32 × 10<sup>5</sup> CFU/ml and in experiment 2 of 6.0 × 10<sup>6</sup> CFU/ml, the *csa* gene real-time PCR detected *C. septicum* with a limit of detection of 63.2 CFU/ml and 60.0 CFU/ml, respectively (Fig. 2).

After establishing that *csa* real-time PCR was a sensitive method in detecting *C. septicum* we screened the stool obtained from 353 patients (192 inpatient samples, 161 community samples). The results for all the samples were negative. While it is possible that we failed to detect *C. septicum* due to either technical errors or levels below the limit of detection, we have used similar methods successfully to detect *C. difficile* in human stools (data not shown). In addition, we performed stool culture for each of these 353 samples on TCCFA (taurocholate cycloserine cefoxitin fructose agar) plates and failed to detect growth of either *C. difficile* or *C. septicum*, although the latter does not grow well on TCCFA. Because of poor sensitivity for TCCFA culture for *C. septicum*, we took additional steps to look for this bacterium. For example, for the six original stool samples from which the DNA yielded the lowest Ct values (39, 36, 36, 36, 38, 35.5 cycles), we re-streaked the original stool onto RCM plates (which support *C. septicum* growth) and no detectable growth of *C. septicum* was observed. Lastly, we failed to detect evidence of *C. septicum* carriage in a Basic Local Alignment Search Tool (BLAST) search of a published *C. septicum* 16S rRNA gene sequence [18] against a collection of partial 16S rRNA gene sequences generated from the stool samples of 338 fecal samples (155 asymptomatic, community samples, 89 diarrheal samples negative for *C. difficile*, and 94 diarrheal samples positive for *C. difficile*) [19]. Those 16S rRNA gene sequence data were used in a separate microbiome study but included 169 of the subjects investigated in the present report [19].

In summary, the point prevalence of *C. septicum* carriage in our geographic region is low. These data suggest that *C. septicum* is not a normal commensal in humans but an opportunistic pathogen whose capacity to cause disease likely reflects the coincidental occurrence of transient carriage and an enhanced host

***C. septicum* alpha-toxin (*csa*) real-time PCR**

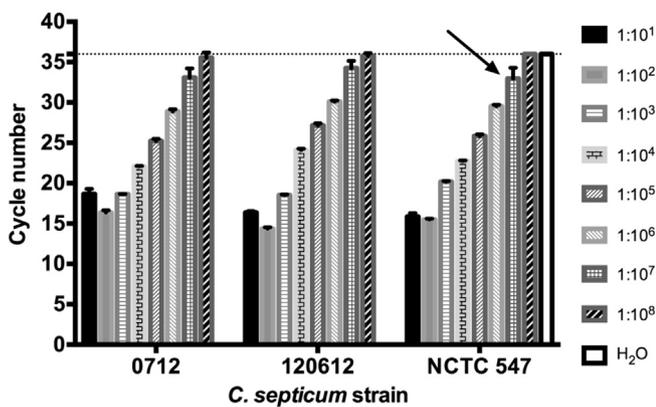


Fig. 1. NCTC 547 had the lowest initial concentration among the three *C. septicum* strains, 172 ng/μl. The arrow marks the column corresponding to the Ct value for the lowest concentration detected (10<sup>-7</sup> dilution) by the *csa* real-time PCR assay. The dotted line at cycle number 36 marks the negative control Ct value.

***C. septicum*-spiked stool real-time PCR**

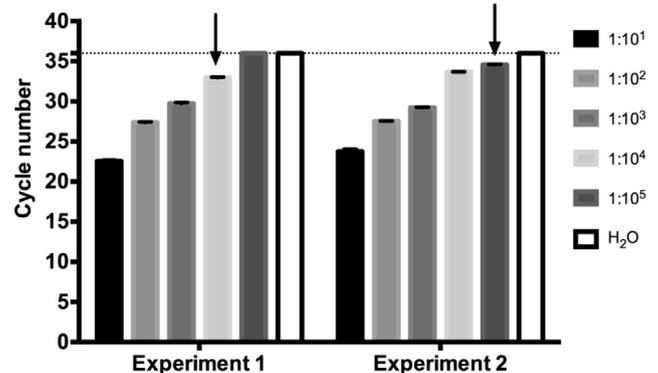


Fig. 2. *Csa* real-time PCR assay detected *C. septicum* in stool spiked with 10<sup>-4</sup> and 10<sup>-5</sup> dilution in experiment 1 and 2, respectively – both marked with an arrow. The dotted line at cycle number 36 marks the negative control (H<sub>2</sub>O) Ct value.

susceptibility (due to risk factors such as malignancy or recent chemotherapy). Future research efforts with larger patient populations followed prospectively are likely to be needed if to identify risk factors for transient carriage of this anaerobic pathogen. It also remains an open question whether there would be measurable benefit to screening for *C. septicum* carriage in patients who are most susceptible to invasive disease, and treating such patients with antibiotics directed at this potential pathogen.

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### Conflict of interest disclosure

Dr. Aronoff has served as an advisor for the Gut Check Foundation, a non-profit organization that promotes education and research into the prevention and treatment of *C. septicum* infections.

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