Gut Pathogens

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Evaluation of a rapid fluorescence immunoassay for detecting *Campylobacter* antigens in stool samples

Lucie Bénéjat[1], Astrid Ducournau[1], Juliette Gebhart[1], Emilie Bessede[1,2], Juergen Becker[3], Marine Jauvain[1,2] and Philippe Lehours[1,2*]

Abstract

Background The species most frequently causing campylobacteriosis are *Campylobacter jejuni* and *Campylobacter coli*, followed by *Campylobacter fetus*, *Campylobacter upsaliensis*, and *Campylobacter lari*. Although polymerase chain reaction (PCR) can be used to detect *Campylobacter* DNA in stool samples, PCR assays are often validated for *C. jejuni* and *C. coli* only, and coproculture results can take several days to receive. For laboratories that do not have access to PCR technology, rapid antigen tests can be of the utmost importance for early diagnosis of the disease. We evaluated the performance of the Sofia *Campylobacter* Fluorescence Immunoassay (SCFIA) for rapid detection of *Campylobacter* antigens in stool. Methods: In total, 94 frozen and 205 fresh stool specimens were included in retrospective and prospective evaluations, respectively. The linearity of the assay and its limit of detection for different *Campylobacter* species was evaluated using serial dilutions. Cross reactivity to phylogenetically related species was also investigated. The PCR results from the BD MAX Enteric Panel were considered the gold standard. Results: The sensitivity of the SCFIA was 97.87% and 96.88% in retrospective and prospective evaluations, respectively. The specificity was 98.84%. The assay exhibited high linearity in serial dilutions for *C. coli*, *C. jejuni*, *C. armoricus*, *C. ornithocola*, *C. lari*, and *C. upsaliensis*, with correlation coefficients of 0.991–0.999, whereas *C. fetus* was not detected. No cross-reactivity was detected for *Aliarcobacter butzleri*, *Helicobacter cinaedi*, or *Helicobacter pullorum*. The minimum concentration for a positive result at the assay-specific cut-off was 4–17 million CFU/mL. The limit of detection ranged from 10^6 to 10^7 CFU/mL. Conclusion: SCFIA results are highly correlated with PCR results, with no cross-reactivity with phylogenetically related species. The linear correlation between fluorescence and CFU/mL results was strong. The assay's ability to detect antigens of various *Campylobacter* species can aid early diagnosis. However, the inability to detect *C. fetus* must be considered.

Keywords *Campylobacter*, Gastroenteritis, Rapid antigen testing, Reference test

*Correspondence:
Philippe Lehours
philippe.lehours@u-bordeaux.fr
[1]National Reference Centre for Campylobacters & Helicobacters, Bordeaux, France
[2]University of Bordeaux, Inserm, UMR 1312, BRIC, Bordeaux Institute of onCology, 146 Rue Léo Saignat, Bordeaux F-33076, France
[3]QuidelOrtho France Sarl, Paris, France

Background

Campylobacter genus is a major cause of bacteria-induced diarrheal infectious diseases worldwide, with an increasing incidence in both high- and low-income countries [1, 2]. In addition, *Campylobacter spp.* can overcome the gastrointestinal barrier, leading to bacteremia. Blood stream infections by *Campylobacter* spp. accounts for < 1% of *Campylobacter* spp. but are associated with substantial mortality rates of 3–28% [3–5]. In addition, bacteremia caused by *Campylobacter* can lead to complications such as infections in the joints, bones, and soft tissues, as well as vascular infections including mycotic aneurysms, endocarditis, spondylodiscitis, and meningoencephalitis [4, 6–11]. Post-infection complications can include reactive arthritis and Guillain–Barré syndrome [12]. Immunoproliferative small intestinal disease, a type of lymphoma, has been reported in association with *Campylobacter* infections. Notably, *Campylobacter jejuni* has been found in biopsy specimens of patients with this intestinal disease; in these patients, antimicrobial therapy targeting *C. jejuni* has led to rapid remission of the disease [13].

According to the European Centre for Disease Control and Prevention (ECDC), the species that most frequently cause campylobacteriosis in Europe are *Campylobacter jejuni* and *Campylobacter coli*, followed by *Campylobacter upsaliensis*, *Campylobacter lari*, and *Campylobacter fetus* [14]. Most cases of *C. upsaliensis* infections have been reported from the European Union (EU), Australia, Canada, South Africa, and the United States [15]. Cats and dogs are the main reservoirs [16].

In 2021, 129, 960*Campylobacter* cases were reported in the EU, with the majority being *C. jejuni* (88.4%) and *C. coli* (10.1%) [14]. During the severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) pandemic, there was an increase in *C. fetus* cases, with 148 reported in 2021 compared to 130 in 2020 and 122 in 2019 [14]. *Campylobacter fetus* infections have recently been identified as the most common cause of *Campylobacter*-associated bacteremia, leading to secondary tissue infections such as vascular infections and endocarditis (83%), with a mortality rate of up to 25% [10, 11, 17]. The primary reservoirs for *C. fetus* are cattle and sheep, and products from these animals are suspected sources of human infections [18]. In France, *C. fetus* recently caused an outbreak in a rehabilitation center, resulting in significant morbidity among elderly patients [19].

Antibiotic therapy for campylobacteriosis is most effective when started within the first 3 days after symptom onset; it shortens the duration of intestinal symptoms and also reduces the gut population of *Campylobacter* [20]. It is recommended to limit transmission in day-care centers and other places with groups of children [21]. Rapid identification of these bacteria can guide the choice of antibiotic therapy.

Stool culture to detect *Campylobacter* requires a minimum of 48 h and has a sensitivity ranging from 60 to 76% [22, 23]. Although the specificity of coproculture is excellent, its sensitivity is reduced for *Campylobacter* spp. detection. Several culture-independent diagnostic tests are available, providing faster results with better sensitivity and good specificity. Among them, molecular methods such as real-time polymerase chain reaction (qPCR) and enzyme-linked immunosorbent assays (ELISAs) require additional automation, are technically demanding, and are often validated only for *C. jejuni* and *C. coli* [23–25]. Since the 2010s, syndromic PCR formats have become the first diagnostic test of choice for the detection of *Campylobacter* spp., often even replacing coproculture. Immunochromatographic tests are easier to use but have lower reported sensitivity [26–28].

This study evaluated the analytical and clinical performance of the Sofia *Campylobacter* Fluorescence Immunoassay (SCFIA) for the rapid detection of *Campylobacter* antigens in stool specimens from patients with signs and symptoms of infectious gastroenteritis. SCFIA is a new rapid test designed for the detection of *C. jejuni*, *C. coli*, *C. upsaliensis*, and *C. lari* antigens in stool specimens. The test uses advanced immunofluorescence-based lateral flow technology to provide a rapid qualitative result within 15 min.

Materials and methods

SCFIA evaluation

This study was conducted between July and November 2023 at the French National Reference Centre for *Campylobacter* and *Helicobacter* (NRCCH) located in the Bacteriology Laboratory at the University Hospital of Bordeaux.

Testing for *Campylobacter* antigens was conducted using the SCFIA (QuidelOrtho Corp., San Diego, CA, USA), for the detection of *C. jejuni*, *C. coli*, *C. upsaliensis*, and *C. lari* antigens in stool specimens. A proprietary algorithm calculates a specimen over cut-off (S/CO) value, such that S/CO ≥ 1 indicates a positive result, and S/CO < 1 indicates a negative result. The S/CO value is an indicator of the ability of the assay to bind antigens of *Campylobacter* species and the antigen content of the specimen. All tests based on the SCFIA were conducted according to the manufacturer's instructions [29]. Frozen or cooled specimens were brought to room temperature and mixed well before SCFIA testing.

A prospective evaluation was conducted using 205 fresh stool specimens sex ratio, 1.05; mean age, 37 ± 32 years) referred for testing for gastrointestinal infectious pathogens. Specimens were transported at 4 °C in Cary–Blair medium (FecalSwab, Copan, Italy) prior to testing.

The samples were plated on Campylosel (bioMérieux, Marcy l'Étoile, France) and incubated for 3 days at 36 °C in jars using an Anoxomat microprocessor (Mart Microbiology, B.V. Lichtenvoorde, The Netherlands) to create a microaerobic atmosphere (80–90% N_2, 5–10% CO_2, and 5–10% H_2). Subsequently, bacteria were identified via matrix-assisted laser desorption ionization–time of flight mass spectrometry (Bruker, 2023 library) as previously described [30]. For molecular detection, 50 µL of each sample was tested on the BD MAX Enteric Bacterial Panel, which includes targets for the detection of *C. jejuni* and *C. coli* [31, 32]. Among these 205 specimens, 173 tested negative in culture and PCR, and 32 were positive (27 *C. jejuni*, 4 *C. coli*, and 1 mixed infection of *C. jejuni* and *C. coli*). We also conducted a retrospective analysis using 94 frozen specimens collected in Cary–Blair medium between 2020 and 2021. These were aliquoted upon reception into tubes that had never previously been defrosted, and stored at − 80 °C. They all tested positive in culture and BD MAX PCR for *Campylobacter* (83 *C. jejuni*, 11 *C. coli*).

Positivity for *Campylobacter* in BD MAX PCR was considered the gold standard.

Linearity, limit of detection, and reactivity to other species

The linearity of the assay S/CO values and limit of detection (LOD) for *C. jejuni*, *C. coli*, *C. upsaliensis*, and *C. lari* were evaluated using serial dilutions. To assess reactivity to other *Campylobacter* species not declared by the manufacturer, *C. armoricus* (CCUG 73571T), *C. fetus* (ATCC 27374), and *C. ornithocola* (CECT 9147) were included in the serial dilutions because these species can also cause human gastroenteritis. The phylogenetically related species *Aliarcobacter butzleri* (ATCC 49616), *Helicobacter cinaedi* (CCUG 18818T), and *Helicobacter pullorum* (CCUG 33837T) were included to investigate potential cross-reactivity that could result in false-positive test results.

To establish the serial dilutions, well-characterized specimens with known species were grown on blood agar plates under microaerobic conditions. Subsequently, species identity was verified via MALDI-TOF. The grown cultures were used to create stock solutions for each species in Cary–Blair medium for a subsequent serial dilutions. The established stock solutions had the following concentrations in colony-forming units (CFUs) per mL: 3×10^8 (*C. armoricus*), 4.2×10^8 (*C. coli*), 1.2×10^8 (*C. fetus*), 3.9×10^8 (*C. jejuni*), 4.5×10^8 (*C. lari*), 1.7×10^8 (*C. ornithocola*), 4.5×10^7 (*C. upsaliensis*), 1.4×10^7 (*A. butzleri*), 5.4×10^7 (*H. cinaedi*), and 5.4×10^7 (*H. pullorum*). Stock solutions were diluted by 1:10, 1:100, 1:1000, 1:10,000, and 1:100,000. Each dilution was tested with the SCFIA to estimate the LOD of the assay. The linearity of the assay was evaluated by correlating the S/CO values with the individual dilutions.

Results

Accuracy

In the prospective evaluation of 205 specimens, the rate of positive agreement between the SCFIA and the gold standard was 96.88% (Table 1). The rate of negative agreement was 98.84%. In the retrospective evaluation, the positive agreement rate between the SCFIA and the gold standard was 97.87%. Overall, the positive agreement rate between the SCFIA and the gold standard was 97.62 (Table 1).

Three specimens that tested positive in culture (3 *C. jejuni*, 1 from the prospective samples, and 2 from the retrospective samples) and PCR but negative in the SCFIA were also positive in an ELISA (RIDASCREEN *Campylobacter*, R-Biopharm, Darmstadt, Germany). Two specimens from the prospective samples that tested negative in culture but positive in the SCFIA were also positive in PCR (Table 1).

Linearity and reactivity to other *Campylobacter* species

In the serial dilutions established for different *Campylobacter* species, the SCFIA showed the expected reactivity to *C. coli*, *C. jejuni*, *C. lari*, and *C. upsaliensis*, as described by the manufacturer. However, it also demonstrated reactivity to *C. ornithocola* and *C. armoricus*. The last dilution that provided a positive test result (S/CO > 1)

Table 1 Positive, negative, and overall agreement between SCFIA and the gold standard

Comparison vs. gold standard		Prospective samples		Retrospective samples		Prospective + retrospective samples	
		pos (*n*)	neg (*n*)	pos (*n*)	neg (*n*)	pos (*n*)	neg (*n*)
SCFIA	**pos (*n*)**	31	2	92	NA	123	2
	neg (*n*)	1	171	2	NA	3	171
Positive agreement (95% CI)		96.88 (82.00–99,84)		97.87 (91.79–99.63)		97.62 (92.67–99.38)	
Negative agreement (95% CI)		98.84 (95.45–99.80)		NA		98.84 (95.45–99.80)	
Overall agreement		98.54		97.87		98.33	

CI, confidence interval; *n*, total number; NA, not applicable; neg, negative; pos, positive

Gold standard refers to positivity in the BD MAX polymerase chain reaction test

Table 2 SCFIA S/CO and CFU/mL values of serial dilutions for *Campylobacter* species

Species	Dilution	CFU/mL	S/CO	Species	Dilution	CFU/mL	S/CO
C. armoricus	0	3.0×10^8	29.826	***C. jejuni***	0	3.9×10^8	27.456
	1:10	3.0×10^7	4.815		1:10	3.9×10^7	4.109
	1:100	3.0×10^6	0.700		1:100	3.9×10^6	0.425
	1:1,000	3.0×10^5	0.125		1:1,000	3.9×10^5	0.000
	1:10,000	3.0×10^4	0.0720		1:10,000	3.9×10^4	0.0437
C. coli	0	4.2×10^8	19.208	***C. upsaliensis***	0	4.5×10^7	26.085
	1:10	4.2×10^7	2.529		1:10	4.5×10^6	3.963
	1:100	4.2×10^6	0.222		1:100	4.5×10^5	0.434
	1:1,000	4.2×10^5	0.135		1:1,000	4.5×10^4	0.120
	1:10,000	4.2×10^4	0.0000		1:10,000	4.5×10^3	0.0373
C. lari	0	4.5×10^8	24.655	***C. fetus***	0	1.2×10^8	0.000
	1:10	4.5×10^7	4.717		1:10	1.2×10^7	0.138
	1:100	4.5×10^6	0.154		1:100	1.2×10^6	0.140
	1:1,000	4.5×10^5	0.000		1:1,000	1.2×10^5	0.138
	1:10,000	4.5×10^4	0.0000		1:10,000	1.2×10^4	0.1007
C. ornithocola	0	1.7×10^8	28.297	**Average over all species and dilution series**	0	3.0×10^8	25,92
	1:10	1.7×10^7	3.922		1:10	3.0×10^7	4,01
	1:100	1.7×10^6	0.438		1:100	3.0×10^6	0,40
	1:1,000	1.7×10^5	0.056		1:1,000	3.0×10^5	0,07
	1:10,000	1.7×10^4	0.0000		1:10,000	3.0×10^4	0,03

S/CO values ≥ 1 indicate positive test results; S/CO values < 1 indicate negative results. Data for the 1:100,000 dilution are not shown

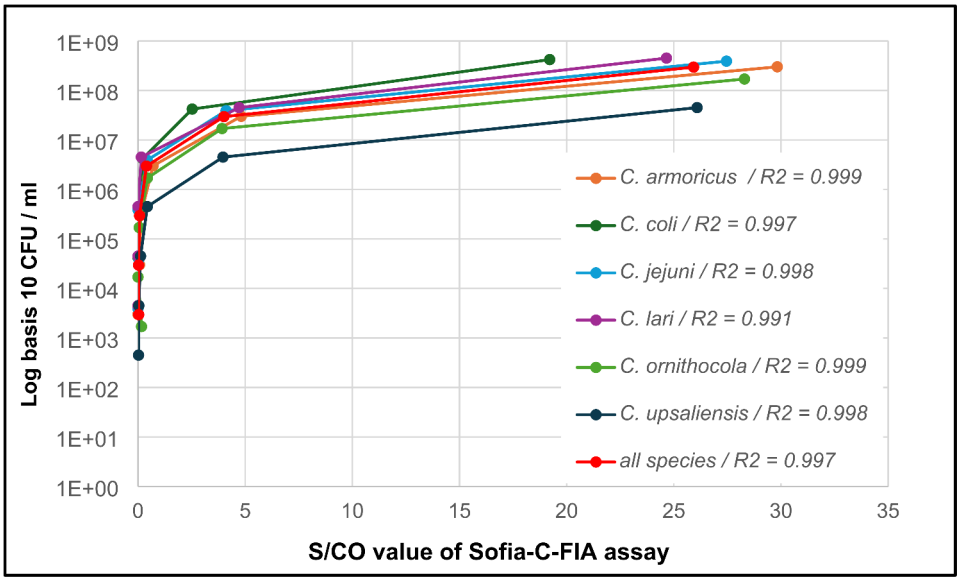

Fig. 1 Linear trend and correlation coefficients (R^2) between Sofia *Campylobacter* Fluorescence Immunoassay (SCFIA) specimen cut-off (S/CO) values and the number of colony-forming units (CFU)/mL per dilution step

for all species was 1:10. The S/CO values for positive results at the 1:10 dilution were 2.53 for *C. coli* and 4.82 for *C. armoricus*. The bacterial concentrations for positive results at the 1:10 dilution were 4.5×10^6 CFU/mL for *C. upsaliensis* and 4.5×10^7 CFU/mL for *C. lari*. Overall, the average S/CO at the 1:10 dilution was 4.01, correlated with a concentration of 3.0×10^7 CFU/mL.

The test results for *C. fetus* were negative even in the undiluted specimens at the highest concentration, with S/CO values near zero, indicating that the assay antibodies do not have any affinity to bind *C. fetus* antigens or to detect this species even at high concentrations (Table 2).

The S/CO values were strongly correlated with the estimated number of bacterial copies per mL for each *Campylobacter* species, with correlation coefficients (R^2) ranging from 0.991 for *C. lari* to 0.999 for *C. ornithocola* and *C. armoricus*. Across all species, the R^2 was 0.9971 (Fig. 1). Given the high linear correlation observed

Table 3 Estimated minimum bacterial concentrations (CFU/mL) required in SCFIA for a positive test result (S/CO ≥ 1)

Species	Linear regression y = CFU/mL x = S/CO	Estimated CFU/mL for S/CO = 1
C. armoricus	$y = 1 \times 10^7 x - 4 \times 10^6$	6.00×10^6
C. coli	$y = 2 \times 10^7 x - 3 \times 10^6$	1.70×10^7
C. jejuni	$y = 1 \times 10^7 x - 4 \times 10^6$	6.00×10^6
C. lari	$y = 2 \times 10^7 x - 6 \times 10^6$	1.40×10^7
C. ornithocola	$y = 6 \times 10^6 x - 2 \times 10^6$	4.00×10^6
C. upsaliensis	$y = 2 \times 10^6 x - 534{,}225$	1.47×10^6
All species	$y = 1 \times 10^7 x - 3 \times 10^6$	7.00×10^6

between S/CO values measured using the SCFIA and the bacterial concentration in the dilutions for all *Campylobacter* species, a linear regression analysis was conducted to estimate the number of CFU/mL needed to reach a positive test result (S/CO ≥ 1). The estimated minimum concentration in CFU/mL at the test-specific S/CO cut-off of 1 was 1.47×10^6 for *C. upsaliensis*, while the maximum was 17×10^6 CFU/mL for *C. coli* (Table 3).

Reactivity to phylogenetically related species

The phylogenetically related species *A. butzleri*, *H. cinaedi*, and *H. pullorum* were included in the serial dilutions with initial concentrations of 1.4×10^7 for *A. butzleri* and 5.4×10^7 for both *H. cinaedi* and *H. pullorum*. The SCFIA test provided negative results for all three species, even at the highest concentration. The measured S/CO values were zero for all three species at the highest concentration, indicating that the SCFIA test has no potential to cross-react with these phylogenetically related species.

Discussion

The performance and value of *Campylobacter* antigen detection in stool samples have been described in numerous reports [33]. To our knowledge, this study presents the first independent evaluation of the performance of the SCFIA. Stool antigen tests have shown variable performance, perhaps due to intrinsic differences among the tests or the reference methods used in different studies. Culture methods are particularly known to lack sensitivity for *Campylobacter* detection compared to PCR methods. We assessed the clinical performance of the SCFIA using stool specimens confirmed to be positive via PCR, in both prospective and retrospective analyses.

The advantage of the SCFIA test kit is its automated reading, which eliminates operator influence. This is particularly beneficial for samples with low positivity, where immunochromatographic tests can be misinterpreted by users.

Our retrospective evaluation included 299 samples, of which 126 (42.1%) were positive for *Campylobacter*: 110 *C. jejuni* (87.3%), 15 *C. coli* (11.9%), and 1 for both, *C. jejuni* and *C. coli* (0.8%). This distribution closely matches data reported by the ECDC at the European level. According to the ECDC, 88.4% of confirmed infections in 2021 were caused by *C. jejuni*, followed by *C. coli* at 10.1% [14].

The linear correlation between S/CO values and approximated CFU/mL values was high across all individual serial dilutions and species, with correlation R^2 values ranging from 0.991 (lowest for *C. lari*) to 0.999 (highest for *C. armoricus* and *C. ornithocola*). All serial dilutions included specimens with concentrations below and above the S/CO cut-off value of 1 and the LOD of the assay. The strong correlation between S/CO values and bacterial concentrations, spanning a wide range and including specimens around the assay cut-off and LOD, is a key indicator of the test's reliability, particularly for borderline specimens. Infected humans usually excrete 10^6 to 10^9*C. jejuni* per gram of stool [34]. This test is therefore sufficiently sensitive to detect *C. jejuni* and *C. coli* in human stool specimens.

Linear regression estimated bacterial concentrations between 1.47×10^6 and 17×10^6 CFU/mL for different *Campylobacter* species at the S/CO cut-off of 1, with the exception of *C. fetus*. This result indicates that the ability of the assay to detect *C. jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, *C. armoricus*, and *C. ornithocola* is nearly equivalent. Such near-equivalence in human tests has not previously been described. For example, a study that evaluated the ProsPect *Campylobacter* immunoassay (Remel, Lenexa, KS, USA) reported higher detection limits for *C. jejuni* and *C. coli* than for other *Campylobacter* species that were also detectable [35]. The ability of some tests to detect *C. upsaliensis* has been previously described [36–40]. Generally, these studies reported a 1- to 10-fold lower sensitivity for *C. upsaliensis* compared to *C. jejuni* [38, 39], which is not the case with the SCFIA.

In this study, the SCFIA showed good reactivity for *C. ornithocola* and *C. armoricus*, in addition to species listed in the manufacturer's instructions (*C. jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis*). This finding is not surprising, as *C. ornithocola* and *C. armoricus* were described in 2017 and 2019 as members of the *C. lari* group [41, 42] due to their strong phylogenetic relationship. Thus, the reactivity of the SCFIA was expected due to antigen similarity between these two species and *C. lari*. The ability of certain kits to detect *C. lari* has been described; for example, Kawatsu et al. [37] reported a *Campylobacter* immunochromatographic assay that detects a 15-kDa cell surface protein of *C. jejuni*, and Regnath et al. [43] reported similar results using the RIDA QUICK and RIDASCREEN *Campylobacter* kits (R-biopharm). The clinical significance of this finding is likely limited. Both *C. ornithocola* and *C. armoricus* are rarely detected in stool specimens

from patients with gastroenteritis because these species are not detectable by the syndromic PCR formats marketed worldwide. They are also difficult to distinguish from *C. lari* via MALDI-TOF due to their phylogenetic proximity [41, 42]. In 2021, we detected *C. ornithocola* and *C. armoricus* in 2 and 4 of 8,709 strains sent to our reference center, respectively. In 2022, 4 of 8,971 strains tested at our reference center corresponded to *C. ornithocola*, whereas *C. armoricus* was not detected. These data indicate that infections with these two species are very rare [44].

However, we acknowledge the absence of detection of *C. fetus*, which is the third most common *Campylobacter* species isolated from campylobacteriosis specimens in France (NRCCH data available on www.cnrch.fr). *Campylobacter fetus* can cause invasive infections in elderly or immunocompromised patients [5]. Unfortunately, no kit on the market currently detects this species, making this detection gap common. Investigating the absence of cross-reactivity with closely related bacteria such as *Aliarcobacter* and enterohepatic *Helicobacter* in the kit was crucial in developing and evaluating a new test, as has already been achieved by other research teams [36, 37].

Conclusion

SCFIA is a rapid, accurate antigen test that could be of great utility to severely ill hospitalized patients who could benefit from early targeted antimicrobial therapy. Its capacity to detect the main species responsible for campylobacteriosis is of major interest for clinical use.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13099-025-00686-4.

Supplementary Material 1

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Author contributions
PL supervised the study. JB, MJ, EB, and PL analyzed the data and drafted the manuscript. LB, AD, JG performed the experiments. All authors interpreted the data. All authors critically revised the manuscript for important intellectual content.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All diagnostic methods are routinely performed at our institution. Therefore, informed consent was not requested from patients to use their stool samples. However, to ensure subject anonymity, all identifiable patient data were removed from the present study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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