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Review

Advanced Methods for Isolating from and Confirming *Campylobacter* spp. in Milk and Dairy Products: Review

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Abstract

Campylobacter spp. are a type of microaerophilic bacteria that cause human foodborne illnesses worldwide. Among the various types of *Campylobacter* spp., *Campylobacter jejuni* and *Campylobacter coli* account for 90% of foodborne campylobacteriosis. Generally, poultry meats are known to be a primary cause of campylobacteriosis; however, several other types of foods have also been reported to cause campylobacteriosis. Particularly, raw milk has been directly linked to *Campylobacter* infections among many foodborne illnesses, and cases of campylobacteriosis caused because of the ingestion of unpasteurized raw milk have been recorded worldwide. This review reports (1) general information, history, and nomenclature of *Campylobacter* spp., (2) epidemiology of *Campylobacter* spp., (3) detection of *Campylobacter* spp. from foods including milk and dairy products, and (4) review of methods for controlling the growth *Campylobacter* spp.

Keywords

Campylobacter, milk, dairy foods, culture method, rapid detection

Introduction

Campylobacter spp. is the most frequent causative agent of foodborne illness worldwide, and *C. jejuni* and *C. coli* together account for the majority of food-borne campylobacteriosis among all *Campylobacter* spp. [1-3]. Generally, poultry meats in particular have been regarded a primary cause of campylobacteriosis among various types of foods [4]. However, other types of food such as ground beef, water, oyster, egg, vegetable and milk have also been reported as a cause of *Campylobacter* illness [2,4,5]. Above all, unpasteurized raw milk is a well-known cause of *Campylobacter* outbreaks, with numerous reported outbreaks from the UK, Poland, and elsewhere [5-7]. Raw milk may is contaminated with the *Campylobacter* spp. in a variety of routes, because *Campylobacter* spp. are ubiquitous in cow and dairy farms [5]. For example, uncleaned milking machines, mastitis (cow's udder disease), and fecal contamination of the reservoir could directly affect the outbreaks of *Campylobacter* infection [5-7]. Also, cross contamination of ready to eat foods during food preparation as well as direct contact with animals have been identified [6,7].

The detection and identification of *Campylobacter* spp. is somewhat difficult because of its long incubation period and unique culture requirements such as microaerobic conditions [8]. Various rapid and sensitive detection methods such as immunological

detection and PCR assay have recently been developed to overcome the drawbacks [2]. One of which is the VIDAS apparatus which uses automated ELISA technique [2]. This automated immunoassay could be an effective screening tool, since it saves time and labor [9]. However, the kit has not been fully validated for its efficiency and accuracy in detecting *Campylobacter* spp. in various foods [2].

Although a variety of rapid methods have been developed to detect *Campylobacter* spp. in food samples, selective agar culture is the most commonly used method [10]. *Campylobacter* selective agars being used in current standard culture methods are classified into two groups. One group uses animal blood as supplement, which includes Skirrow agar, Blaser agar, Campy-Cefex agar, and Preston agar. On the other hand, blood free media group includes mCCDA, Karmali agar, and cefoperazone amphotericin teicoplanin (CAT) agar [11]. Of various *Campylobacter* selective broths, Bolton broth is the enrichment broth most commonly used by many food authorities such as the United States Food and Drug Administration (US FDA), the United States Department of Agriculture Department- Food Safety and Inspection Service (USDA FSIS), and the International Organization for Standardization (ISO) [12-14]. To exclude the growth of competing flora, the broth contains a variety of antimicrobial agents such as cefoperazone, vancomycin, and trimethoprim [15]. Cefoperazone, a third-generation cephalosporin, is the most commonly used antibiotic supplement in *Campylobacter* agar and broths. Most *Campylobacter* selective media such as mCCDA, Campy-Cefex agar, Bolton broth, and Karmali agar that are frequently used by food authorities are supplemented with a high concentration of cefoperazone [16].

However, cefoperazone resistance in bacteria has recently become more widespread, making it difficult to isolate *Campylobacter* spp. from raw poultry meat [17-20]. ESBL is an enzyme produced by bacteria that renders cephalosporin resistance [21]. In many countries, ESBL producing *E. coli* strains resistant to cefoperazone have been frequently isolated from raw chicken [22]. Previous studies have reported that extended-spectrum beta-lactamases (ESBL)-producing *E. coli* may overgrow on mCCDA and Campy-Cefex agar media supplemented with cefoperazone, making it difficult to differentiate and isolate suspected *Campylobacter* colonies [17-20]. Therefore, elimination of ESBL-producing *E. coli* by using a novel approach could tremendously increase the sensitivity and selectivity of *Campylobacter* selective agar [2].

Increasing resistance of indigenous poultry flora to antibiotic agents in selective media has necessitated the development of improved culture methods. Because of their small width (0.2-0.8 μm), length (0.5-5.0 μm), corkscrew-like motility, and spiral morphology, *Campylobacter* spp. can pass through 0.45-0.8 μm filters [23]. Membrane filtration techniques employing cellulose nitrate, cellulose triacetate, and cellulose acetate filters have been used to exclude competing flora during the isolation of *Campylobacter* spp. [23]. The direct application of membrane filters to the surface has been used to exclude unwanted microflora during selective isolation of *Campylobacter* spp. [24]. However, membrane filtration is associated with complications, such as a lengthy filtration time, drying of the agar plate, and mishandling, thus leading to contamination.

1. General information, history, and nomenclature for *Campylobacter* spp.

Members of the *Campylobacteraceae* family are gram-negative bacteria that have a curved or spiral rod shape, a small width (0.2–0.8 μm) and length (0.5–5.0 μm), and corkscrew-like motility [25]. The corkscrew-like motility is driven by a single, polar unsheathed flagellum at one or both ends of the bacteria. In stressed environments, these bacterial cells may form spherical or coccoid bodies that are viable but non-culturable [26]. Cellular energy is obtained via amino acids or tricarboxylic acid cycle intermediates, rather than by carbohydrate metabolism [25]. The optimum growth temperature is 37℃ to 42℃ under microaerobic conditions [26].

McFadyean & Stockman [27] isolated *Vibrio*-like bacteria from an aborted ovine fetus in 1913. Smith [28] and Smith & Taylor [29] also observed spiral bacteria belonging to the same species in an aborted bovine fetus in 1918, and designated the bacteria *Vibrio fetus*. The microorganisms can cause sporadic abortions or infectious infertility in cattle. In 1927, Smith & Orcutt [30] isolated another *Vibrio*-like bacteria from the feces of cattle suffering from diarrhea. Jones et al. [31] described the association between the bacteria and bovine diarrhea and proposed the name *V. jejuni*. Doyle [32] isolated another *Vibrio*-like bacteria from porcine feces in 1944 and designated the bacteria as *V. coli*. Sebald & Veron [33] renamed *V. fetus* and *V. bubulus* as *C. fetus* and *C. bubulus*, respectively in 1963, due to their unique characteristics, including low DNA base compositions, microaerobic growth requirements, and nonfermentative metabolism. Véron and Chatelain [34] reported a more comprehensive study in 1973 regarding the taxonomy of *Vibrio*-like microorganisms growing under microaerobic condition. They classified four distinct species in the *Campylobacter* genus: *C. coli*, *C. fetus*, *C. jejuni*, and *C. sputorum*. Several other novel *Campylobacter* species were found by researchers in the 1980s, including *C. cinaedi*, *C. concisus*, *C. cryaerophila*, *C. fennellia*, *C. hyointestinalis*, *C. lari*, *C. mustelae*, *C. nitrofigilis*, and *C. pyloridis* [35]. *C. pyloridis* was proposed as a human gastric *Campylobacter* species in 1984. *Campylobacter* taxonomy was revised by Goodwin et al. [36], who proposed the name of a novel genus, *Helicobacter*, to include *C. pylori* and *C. mustelae*. Vandamme et al. [37] proposed a complete revision of the taxonomy and nomenclature in 1991. At present, there are various *Campylobacter* species that are validly named as follows: *C. avium*, *C. canadensis*, *C. coli*, *C. concisus*, *C. cuniculorum*, *C. curvus*, *C. fetus*, *C. gracilis*, *C. helveticus*, *C. hominis*, *C. hyointestinalis*, *C. insulaenigrae*, *C. jejuni*, *C. lanienae*, *C. lari*, *C. mucosalis*, *C. peloridis*, *C. rectus*, *C. showae*, *C. sputorum*, *C. subantarcticus*, *C. upsaliensis*, and *C. volucris* [38].

2. Epidemiology of *Campylobacter* spp.

For decades, *Campylobacter* spp. have been regarded as a cause of septic abortion in cattle and sheep and of diarrhea in cattle. However, they also have been recognized as a pathogen causing food poisoning in humans [25]. Specifically, *C. jejuni* and *C. coli* are the main causes of campylobacteriosis in humans [3]. Among many foods including milk and dairy foods, animal origin foods, such as poultry meat and poultry products,

play a major role in *Campylobacter* infections in humans. Most *Campylobacter* infections are limited to sporadic cases or small family outbreaks [25]. Previous epidemiological studies found a significant association between campylobacteriosis and the consumption of raw or undercooked poultry meat [4]. However, other types of foods must be considered as potential sources of infection. In addition to poultry, *Campylobacter* spp. also has been detected in raw milk, pork, beef, lamb, and seafood [3,4]. In a retrospective cohort study conducted in the United Kingdom, Evans et al. [39] found that vegetable salads were the second-highest risk factor for *Campylobacter* infection after chicken. According to population-based cohort studies conducted in the United Kingdom and the Netherlands, the incidences of food poisoning caused by *Campylobacter* spp. in the United Kingdom and the Netherlands were estimated to be 9.3/1000 person-years (2008–2009) and 5.8/1000 person-years (2009), respectively [40]. In 1982, the Centers for Disease Control and Prevention (CDC) initiated national surveillance of *Campylobacter* spp. through the Public Health Laboratory Information System [2]. In the USA, one out of 30.3 cases is reported by Foodborne Diseases Active Surveillance Network (FoodNet) sites. The national incidence of *Campylobacter*-related food poisoning in the USA was 1.3 million cases in 2006 or 4.4/1000 person-years [40]. In 2014, the FoodNet reported that the number of patients and incidences of campylobacteriosis per 100,000 population were estimated to be 6,621 and 13.82, respectively [41] (Table 1).

Like other foodborne pathogens, *Campylobacter* spp. cause acute diarrheal enteritis in the intestinal tract with clinical manifestations [25]. *Campylobacter*-related illness can be definitively diagnosed by isolating the pathogen from feces. No clear differences in symptoms are observed between infections caused by *C. jejuni* and *C. coli* [25]. Acute inflammatory enteritis is an essential lesion in campylobacteriosis, which commonly extends down the intestine to affect the colon and rectum [25]. The infectious dose of *Campylobacter* spp. is known to be low, and exposure to 500 colony-forming units

Table 1. Number of cases of culture-confirmed bacterial and laboratory-confirmed parasitic infection, hospitalizations, and deaths, by pathogen — Foodborne Diseases Active Surveillance Network, USA, 2013[1)]

Bacterial pathogen	Cases			Hospitalization		Deaths	
	No.	Incidence[2)]	Objective[3)]	No.	%	No.	%
Campylobacter	6,621	13.82	8.5	1,010	15	12	0.2
Listeria	123	0.26	0.2	112	91	24	19.5
Salmonella	7,277	15.19	11.4	2,003	28	27	0.4
Shigella	2,309	4.82	N/A	450	19	3	0.1
STEC O157	552	1.15	0.6	210	38	2	0.4
STEC non-O157	561	1.17	N/A	76	14	2	0.4
Vibrio	242	0.51	0.2	55	23	2	0.8
Yersinia	171	0.36	0.3	55	23	4	2.3

Table from Crim et al. with [41].
1) Data for 2013 are preliminary.
2) Per 100,000 population.
3) Healthy People 2020 objective targets for incidence of *Campylobacter, Listeria, Salmonella*, STEC O157, *Vibrio*, and *Yersinia* infections per 100,000 population.
N/A, not available; STEC, Shiga toxin–producing *E. coli*.

(CFUs) *Campylobacter* spp. can cause food poisoning in humans [42]. By investigating disease spread originating from 17 point-source outbreaks, the mean incubation period before campylobacteriosis developed was estimated 3.2 days, with a range of 8 h to 8 d [25]. The onset of enteritis is abrupt with abdominal pains followed by diarrhea. Approximately 30% of patients suffer from nonspecific influenza-like symptoms, such as fever, headache, dizziness, and myalgia [25]. In diarrheal stag, profuse, watery, bile-stained, and prostrating diarrhea has been observed. Although many patients experience nausea, only ~15% of patients report vomiting [25]. After approximately 3 to 4 days into the illness, the diarrhea begins to subside and the symptoms of patients begin to lessen, although abdominal pain may persist for a few more days afterward [25]. Minor relapses have been found in 15% to 25% of patients who visited a hospital following the development of symptoms [43]. Fatal outcomes or serious complications are generally limited to elderly or immunocompromised individuals or those with some other disease [44]. In some instances, *C. jejuni* has been found to be an etiological agent in the development of Guillain-Barre syndrome [25].

3. Detection of *Campylobacter* spp. from foods including milk and dairy foods

A major obstacle in *Campylobacter* spp. research, particularly in human medicine, had been the difficulty encountered in isolating these bacteria. In the early 1970s, Butzler et al. [45] applied a filtration method, taking advantage of the small cellular size and the vigorous motility of *Campylobacter* cells to selectively isolate them from stools of humans with diarrhea [45]. The main breakthrough, however, was provided a few years later by Skirrow, who described a selective supplement comprising a mixture of vancomycin, polymyxin B, and trimethoprim that was added to basal growth medium [46]. The development of Skirrow medium was a key advance in the study of thermotolerant *Campylobacter* species. The use of Skirrow medium enabled the successful recovery of thermotolerant *Campylobacter* and therefore provided evidence linking *Campylobacter*-related disease to food contamination, especially of chicken [46]. The selective media commonly used for isolating pathogens from foods, water, and environmental samples were originally derived from media developed for clinical purposes [25]. Fecal samples often contain large numbers of viable *Campylobacter* bacteria, which can be detected by directly plating samples on selective media. The various media used to isolate *Campylobacter* spp. from food samples are normally supplemented with selective or non-selective agents in different combinations and concentrations [25]. Cefoperazone, cycloheximide, amphotericin, trimethoprim, rifampicin, colistin, and vancomycin frequently serve as selective agents [15,25]. Blood, activated charcoal, sodium pyruvate, and ferrous sulfate are commonly added as non-selective agents to *Campylobacter*-isolation media to neutralize the toxic effects of oxygen and light [23]. *Campylobacter* cells are usually grown at 37℃ to 42℃ under microaerobic conditions [26]. Several incubation methods are available to achieve optimal microaerobic conditions, such as growth in CO_2 incubators or anaerobic jars containing microaerobic pouches [23,25].

1) Selective agar

Methods for isolating *Campylobacter* bacteria have been in existence for decades, including the use of selective media. Selective media supplemented with non-selective agents are broadly classified into two groups [2]. One group uses sterile sheep or horse blood as a non-selective supplement and includes Skirrow, Butzler, Campy-Cefex agar, and Preston media [2]. Alternatively, blood-free media have been developed that contain activated charcoal, including mCCD agar, Karmali agar, and CAT agar [11]. Because sterile blood is expensive and readily contaminated, blood-free media containing activated charcoal offers advantages over blood-containing media [47]. Among these selective agars, the Preston, Butzler, Karmali, CampyCefex, and mCCDA agars are most commonly used in standard detection protocols recommended by many food authorities [48]. Corry et al. [15] and Corry and Atabay [23] proposed a detailed formulation of Preston and CCD agar for their studies containing charcoal, cefazolin, and deoxycholate. The antibiotic component of derivative agars has been varied for decades, and in more recent studies, cefazolin has been replaced with cefoperazone [15,23]. Karmali et al. [47] developed a blood-free charcoal-based *Campylobacter* selective agar that effectively supports the growth of *Campylobacter* spp. This selective agar contains charcoal, hematin, cefoperazone, vancomycin, and sodium pyruvate, but not blood products [2]. Campy-Cefex agar is the one of the most commonly used selective media by food authorities such as the USDA FSIS and has facilitated *Campylobacter* isolation in numerous studies with poultry samples [13]. Campy-Cefex agar enables more sensitive quantitative detection of *Campylobacter* isolates in poultry samples compared to other selective agars such as mCCDA, Karmali, and Campy-Line agar [16]. However, some investigators have reported that this agar is may be subject to contamination by non-*Campylobacter* isolates on the agar surface, with competing flora making isolation of the target bacteria difficult [49]. Aspinall et al. [50] developed CAT agar containing 8 mg cefeopearoze and 4 mg of teicoplanin by modifying mCCDA agar. In 1996, Aspinall et al. reported that the CAT agar was superior to mCCDA for isolating *C. upsalienesis* from animal feces [50]. Recently, various *C. jejuni/C. coli* chromogenic plating agars have been introduced into the market, such as the CampyFood (BioMérieux, sa, France), Brilliance CampyCount Agar (Oxoid, Hampshire, UK), CASA (BioMérieux), and R&F *Campylobacter* media (R&F Laboratories, USA). The use of R&F chromogenic media supplied from R&F Laboratories will be recommended as a new method by the US FDA [51]. Ahmed et al. [52] validated the above-mentioned chromogenic media in comparison studies with conventional agars for isolating *Campylobacter* spp. from poultry samples and reported that CASA media showed superior detection ability (Table 2).

2) Selective enrichment broth for isolating *Campylobacter* spp.

Recovering *Campylobacter* isolates from food and environmental samples is difficult because of the complicated incubation conditions required for bacterial growth and because such bacteria grow poorly in artificial environments. Moreover, the number of different *Campylobacter* spp. is often low, and these microorganisms are typically

Table 2. Recovery of *Campylobacter* spp. from naturally contaminated poultry samples

	CCA	mCCDA	CASA	CFA	BCCA
Number of samples analyzed	95	95	95	95	95
With suspect *Campylobacter* spp.[1)]	21	17	19	26	21
With non-*Campylobacter* spp.	68	19	3	61	16
Number of colonies analyzed	100	66	110	102	105
Positive colonies (%)	84 (84%)	60 (90%)	110 (100%)	89 (87%)	92 (88%)
False positive colonies (%)	16 (16%)	6 (10%)	0 (0%)	13 (13%)	13 (12%)

Table from Ahmed et al. with permission of Elsevier [52].
[1)]Based on typical colony morphology of *Campylobacter* spp. on respective media.

stressed in food samples [8]. Thus, sample enrichment for the recovery of trace amounts of sublethally injured cells is generally recommended as an essential step in isolating *Campylobacter* spp. from foods [25]. The incorporation of an enrichment step into *Campylobacter* isolation protocols from food samples facilitates enhanced sensitivity and recoverability [53]. Numerous selective enrichment broths have been proposed in previous studies. Preston broth, Exeter broth, Bolton broth, *Campylobacter* enrichment broth, and Park & Sanders broth have emerged as the most commonly used enrichment broths for isolating *Campylobacter* spp. from foods [2]. Currently, Bolton, Preston, or Exeter broths are most commonly recommended by several food authorities [23]. However, cefoperazone-resistant bacteria such as ESBL-producing *E. coli* have recently become more widespread, making it difficult to isolate *Campylobacter* spp. from poultry and poultry products [2,11,54]. ESBL is an enzyme produced by bacteria that confers cephalosporin resistance [2]. Results from studies by Moran et al. [54] revealed that ESBL-producing *E. coli* grow exponentially in cefoperazone-containing selective enrichment broth and agars such as Bolton broth, Campy-Cefex agar, and mCCDA agar, thereby inhibiting the selective isolation of *Campylobacter* spp.

3) Identification of *Campylobacter* spp.

To identify *Campylobacter* species, biochemical methods such as latex agglutination tests and ELISAs, as well as molecular identification methods such as PCR and multiplex PCR assays, are normally used following the isolation procedure [16,51]. Currently, the USDA FSIS recommends the use of phase-contrast microscopy or a latex agglutination kit to identify suspected isolates [13,51]. As recommended by the US FDA, the suspected colonies should be confirmed by qPCR using the new methodology [51].

4) Standard method for detecting *Campylobacter* spp. in various foods

The 2006 ISO 10272-1:2006 method includes two components for detecting *Campylobacter* spp. in foods. Part 1 is detection involves enrichment plating, and part 2 involves bacterial enumeration following direct plating. Various selective media such as Bolton broth, Preston broth, mCCDA agar, and Preston agar are currently recommended for this protocol [14,51]. Preston broth is recommended in samples having high

background microflora [51]. The USDA FSIS recommends that both qualitative and quantitative detection protocols be performed for the isolation of *Campylobacter* spp. from chicken rinse samples, using Bolton broth for enrichment and Campy-Cefex agar for selective plating [13]. Recently, however, researchers at USDA FSIS have decided to discontinue the qualitative detection in future [13,51]. In contrast, the FDA BAM recommends using Hunt broth for enrichment and mCCDA and Abeyta-Hunt-Bark agar for selective plating [2]. In the New methodology from the US FDA, Bolton broth and R&F media will be used [51].

5) Filtration method for detecting *Campylobacter* spp. in various foods

C. jejuni was first isolated by a filtration technique from human diarrheal stool in 1972 [55]. Membrane filtration has also been used in more recent applications to exclude unwanted microflora during the selective isolation of *Campylobacter* spp. [24]. In addition to the successful recovery of *Campylobacter* spp. in clinical and water samples, filtration has also been incorporated into methods for improved pathogen isolation from food samples [2,56]. In particular, recent studies have established optimal conditions for filtration-based *Campylobacter* spp. isolation and detection in poultry meat samples [2]. Wisessombat et al. [56] developed a onestep *Campylobacter* isolation device incorporating membrane filtration and an enrichment broth for the selective isolation of *Campylobacter* spp. from poultry.

6) Rapid detection methods for detecting *Campylobacter* spp. in various foods

Although conventional culture methods are commonly used as standard methods for isolating foodborne pathogens from foods, these methods are time consuming and labor intensive [57]. Various rapid and sensitive detection methods such as immunoassays and nucleic acid-based isolation methods have recently been developed to overcome these drawbacks [9,16]. Immunoassays can be more effective than conventional culture methods for screening numerous samples quickly, as the antibodies used in immunoassays enable specific isolation of the target pathogen from complicated matrixes, such as food samples [2,16]. With the development of specific polyclonal and monoclonal antibodies targeting *Campylobacter* spp., various immunoassays are in common use for isolating the pathogen [16]. ELISA assays are conducted in microtiter plates and take advantage of specific antigen-antibody interactions to rapidly isolate the pathogen, as demonstrated by their successful use in isolating *Campylobacter* spp. from various samples [58]. Currently, an automated ELISA method is available for detecting *Campylobacter* spp. in food samples (VIDAS *Campylobacter*). The VIDAS *Campylobacter* test has been validated by researchers with various food samples including chicken, ground beef, and vegetable salad [48]. Immunomagnetic separation methods using small magnetic beads coated by specific antibodies are also commonly used to detect *Campylobacter* spp. in food products [2,16]. The Pathatrix® assay employs recirculating immunomagnetic separation and is an automated assay that can be adjusted for large volumes of food sample rinses or enrichment broths [16]. Pathatrix® systems are

available for testing various foodborne pathogens including *Campylobacter* spp. The detection limits of these systems are approximately 1–10 CFU/25 g after the pre-enrichment step [16]. Molecular methods are also used widely for the rapid isolation and confirmation of contaminating foodborne pathogens following enrichment [16]. PCR is one of the most commonly recommended methods for detecting foodborne bacteria. The identification of the genus and species of a bacterium can be determined by amplifying specific nucleic acid sequences [16]. However, PCR is normally affected by food matrices, interference, and PCR inhibitors present in foods, requiring refined DNA extraction methods after the enrichment step [16]. The quantification of bacterial cells and real-time PCR amplification of bacterial nucleic acids can be achieved in real-time PCR assays [16]. Numerous single and multiplex real-time PCR assays have been developed to detect specific gene sequences in various *Campylobacter* species or genera [59]. Commercialized real-time PCR kits including the BAX® System are now widely used by food authorities and research laboratories to detect *Campylobacter* spp. in pre-enrichment samples. Loopmediated isothermal amplification (LAMP) is an alternative PCR method that uses more than one primer set, resulting in highly specific and sensitive detection. Many scientists developed a real-time LAMP method to detect the *hipO* gene of *Campylobacter* spp. in naturally contaminated cattle farm samples. Combined immunoassay and nucleic acid-based methods have also been developed and validated with food testing, a magnetic immuno-polymerase chain reaction assay to detect *C. jejuni* in milk and chicken samples, and a quantitative immunocapture PCR assay to isolate and detect *C. jejuni* in milk samples and chicken skin washes [2]. DNA or RNA aptamers are alternative ligands for antibodies that can also be used for the immunocapture of *Campylobacter* spp. in pure cultures and foods. However, these rapid detection methods have their own limitations, as these are screening methods and are not as well established as culture methods [2].

Conflict of Interest

The authors declare no potential conflict of interest.

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