COMPARISON, VALIDATION, AND OPTIMIZATION OF INTERNAL GENOMIC DNA EXTRACTION PROTOCOL FOR CAMPYLOBACTER SPECIES

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ABSTRACT

Campylobacter remains the leading cause responsible of human gastroenteritis worldwide. The current study aimed to compare the internal DNA extraction protocol with two commercially available kits, using C. jejuni (ATCC® 29428™) and C. coli (ATCC®43478™) and to validate and optimize the internal protocol through artificial contamination and confirmation of Campylobacter spp. from broiler chickens, turkeys, and beef meats samples. The extraction processes were carried out following the internal protocols and the manufacturer's instructions of PureLink™ Genomic DNA Mini Kit and Wizard® Genomic DNA Purification Kit, respectively. The agarose gel electrophoresis system was used to control the DNA quality. After that, forty Campylobacter spp. isolates were confirmed, and finally, an artificial contamination of the aforementioned reference strains at three different concentrations was performed with sterilized minced meats. This is the first work comparing the PureLink™ Genomic DNA Mini Kit and Wizard® Genomic DNA Purification Kit with internal genomic DNA extraction protocol for Campylobacter spp. The results indicated that the internal protocol provided similar efficiency to the two kits. All confirmed isolates were successfully amplified, in which 28 isolates were C. coli and 12 were C. jejuni, as revealed by biochemical tests. A positive amplification was also observed in the three contaminated food matrices, after enrichment, at all examined doses. Except some reactions that were negative at 1 CFU/mL of C. jejuni and C. coli. This was explained by the detection limits of both internal protocol and qPCR. Based on our findings, three crucial steps in determining the extraction of DNA quality of this protocol were amended. Hence, the study highlighted the importance of validating simpler, cheaper and faster DNA extraction protocol, for each laboratory, as part of future risk assessment, control and monitoring programs of Campylobacter frequency required in molecular studies.

Keywords: Campylobacter spp.; DNA extraction; DNA quantity; qPCR; PureLink™ Genomic DNA Mini Kit; Wizard® Genomic DNA Purification Kit

INTRODUCTION

Campylobacter spp. are Gram-negative, microaerophilic, zoonotic bacteria, and widely known by their pathogenic power that cause human gastroenteritis (campylobacteriosis), with an expensive burden charge on public health (Scharff, 2012). Among Campylobacter species, Campylobacter jejuni (C. jejuni) and Campylobacter coli (C. coli) are the most commonly reported and studied isolates (Del Collo et al., 2017; Gahamanyi et al., 2020). Overall, the disease is self-limiting and can be treated by supportive therapy such as maintenance of hydration and electrolyte balance. Nonetheless, in some cases, the infection may lead to autoimmune syndromes (i.e. Guillain–Barré syndrome and Miller Fisher syndrome), especially in high-risk groups (Skarp et al., 2016; Baaboua et al., 2017). The transmission to human of these thermophilic organisms is made through the consumption of contaminated undercooked meats, drinking water, and raw milk (Budge et al., 2020; Gahamanyi et al., 2020).

For preventing and controlling Campylobacter agents to cause such damages, molecular methods have been widely developed into powerful tools for different applications either in clinical microbiology or in monitoring laboratories for better understanding of their transmission, virulence, surviving factors, and antibiotics resistance mechanisms (Bolton, 2015; Han et al., 2019; Liu et al., 2019). Polymerase Chain Reaction (PCR), Pulse Field Gel Electrophoresis (PFGE), Whole Genome Sequencing (WGS) and others are the typical examples of these techniques (Zou et al., 2010; Chon et al., 2018; Joensen et al., 2020). Indeed, the first common and most important stage of all the aforementioned methods is the genomic DNA extraction. Indeed, numerous DNA extraction protocols and kits commercially available have been described for isolating DNA of Gram-positive and Gram-negative bacteria from biological samples, that were sometimes modified to be more compatible to other microorganisms (Freschi et al., 2005; Dal et al., 2018; Ayana et al., 2019). Basically, the DNA extraction procedure of Gram-negative bacteria is carried out according to four known stages (e.g. Cell lysis, lipids and proteins elimination, DNA wash, and elution) (Bazzicalupo and Fancelli, 1997; Wright et al., 2017).

The efficiency of the DNA extraction protocol was established on the basis of sensitivity of protocols towards the type of bacteria, for example, DNA quality, and purity produced (Leite et al., 2014; Fidler et al., 2020). Moreover, many founded PCR-based tests rely on lengthy and expensive methods for isolating the bacterial DNA (Abdelhai et al., 2016). In this context, the present study aimed (1) to evaluate and compare the DNA extraction methods quality yielded from three genomic DNA extraction protocols by using reference strains of Campylobacter, (2) to determine the advantages of the internal DNA extraction protocol in term of rapidness, cost, and efficiency, and eventually (3) to validate and optimize the internal protocol through artificial contamination and confirmation of Campylobacter spp. from broiler chickens, turkeys, and beef meats samples.

MATERIALS & METHODS

Bacterial strains and growth conditions

C. jejuni (ATCC® 29428™) and C. coli (ATCC®43478™) were purchased from American Type Culture Collection and cultured as described in type strains section. Briefly, the pellet of each bacterium was inoculated in 7 mL of Bolton broth (Biolife, Italiana, Milano-Italy) supplemented with 5% (v/v) of defibrinated horse blood and incubated under microaerophilic conditions (5% O2, 10% CO2, 85% N2) at 37 °C for 48 h. A loopful of 10 µL of each broth culture was streaked onto Colombia blood agar plates (Biolife, Italiana, Milano-Italy) and incubated at 37 °C for 48 h. The pure colonies were suspended in Phosphate-Buffered Saline (PBS) solution and homogenized by vortex to obtain a turbidity of McFarland tube No. 1.0. In order to prepare a similar range of strains concentration, 1 mL of C. coli and C. jejuni suspension were aliquoted in microcentrifuge tubes and stored at -20 °C until the used.
Internal DNA extraction protocol

Reagents employed

The reagents employed in this study were prepared according to the recommendation provided by cold spring harbor protocols (CSH website, accessed on 6.12.20).

Internal DNA extraction protocol

Because of the cost and the limited number of reactions provided by commercially available DNA extraction kits, an internal protocol for Gram-negative bacteria within the Regional Laboratory for Analysis and Research (RLAR) in Tangier, Morocco was investigated. The internal protocol was proposed according to several research papers (Chen and Kuo, 1993; Preschi et al., 2005; Green and Sambrow, 2017) with slight modifications. In brief, 1 mL of each pure culture was centrifuged for 3 min at 12 000 rpm. The cell pellet was resuspended and lysed in 200 µL of lysis buffer (40 mM of tris acetate (pH: 7.8); 20 mM of sodium acetate; 1 mM of EDTA, and 1% of SDS) by vigorous pipetting. Briefly, 66 µL was added of 5M sodium chloride (NaCl) and mixed well. The mixture was centrifuged for 5 min at 14000 rpm. The floating solution was transferred into a new vial, and 266 µL of chloroform was added. The mixture was vortexed until a milky solution was formed and centrifuged for 2 min at 14 000 rpm. The aqueous phase containing DNA was transferred in new vial, in which 40 µL of 3M sodium acetate (pH: 5.2) and 600 µL of isopropanol were respectively added and mixed gently by inversion. After that, the solution was centrifuged for 2 min at 14 000 rpm. The pellet was washed by adding 600 µL of 70% of ethanol, and centrifuged at 14000 rpm for 7 min. The pellet obtained was dried at room temperature and finally, the bacterial DNA was eluted in 100 µL of Tris-EDTA (TE) buffer (10 mM of tris-HCl (pH: 8) and 1 mM of EDTA (pH: 8)) and stored at -20 °C.

Comparison of the internal protocol with commercial kits

From the same batch of strains, C. jejuni and C. coli were also extracted, in triplicate, using Pure Link™ Genomic DNA Mini Kit (Invitrogen corporation, Carlsbad, California, USA) and Wizard® Genomic DNA Purification Kit (Promega, cat no. A1125, Madison, USA), following the manufacturer's instructions.

For each DNA extraction protocol, the DNA quality control was checked by using standard agarose gel electrophoresis.20 µL of extracted DNA were loaded in 1% agarose gel containing ethidium bromide (so to have 0.5 µg/mL), and running in Tris-Acetate-EDTA (TAE) running buffer for 45 min at 85V. The gel was visualized immediately, after electrophoresis, by Vilber Lourmat TM ultra-violet trans illuminator (Labindia Instruments, Mumbai, India) at 254 nm wavelength.

Real time PCR

qPCR probes and primers

The extracted DNA was amplified using CadF gene encodes for outer membrane fibronectin-binding protein of C. coli and HipO gene encodes for C. jejuni hppurrate hydrolase. The oligonucleotides primers and probes used in this study, listed in Table 1, have been provided by Oligonucleotide Information, Bio Basic Canada Inc.

DNA amplification

The real-time PCR (qPCR) reaction mixture was evaluated and amended in our laboratory during this study (unpublished data). All qPCR amplification reactions contained, 2 µL of DNA template, 2.5 µL. Gold buffer (X10), 0.4 mM of dNTP, 5 mM of MgCl2, and 2.5U/µL of Taq polymerase. The concentrations of primers were different for each strain, so that 0.4 µM and 0.12 µM of each primer and C. jejuni-specific probe, respectively, for C. jejuni, while 0.8 µM and 0.12 µM of C. coli-specific primers and probe for C. coli. The reaction mixture was completed by Clinical Laboratory Reagent Water, for a total volume of 25 µL.

Thermal cycling conditions

The amplification was performed with the following thermal cycling conditions: initial denaturation at 95°C for 10 min and 45 cycles; in which each cycle consisting of denaturation at 95°C for 15s, annealing at 60°C for 1 min, and followed by elongation at 72°C for 30s. The reaction was conducted in the Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, Foster city, USA) and quantitative results of real-time PCR were assessed on threshold cycle values (Ct). The reaction was considered positive with Ct ≤36 and negative with Ct > 36.

Validation of internal protocol

Confirmation of Campylobacter species

From February to June 2018, in Northern of Morocco, forty suspicious Campylobacter spp. isolates were collected according to the Moroccan standard NM ISO: 10272-1 (2008) from Campylobacter blood base agar Plates (Biolife, Italiana, Milano-Italy) containing 5% defibrinated horse blood and antimicrobial supplement (Polymyxin B, cycloheximide, rifampicin and trimethoprim). The suspected colonies recovered from broiler chikens, turkeys, and beef meats samples, were biochemically confirmed as C. jejuni and C. coli, respectively following the recommended tests in the NM ISO: 10272-1 (2008). The pure isolated strains underwent molecular confirmation using the internal DNA extraction process followed by the qPCR procedure as reported previously (Figure 1).

Table 1 Oligonucleotides probes and primers used for C. jejuni and C. coli (Oligonucleotide Information, Bio Basic Canada Inc.)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Gene</th>
<th>Oligonucleotide</th>
<th>Sequence (5′→3′)</th>
<th>Product Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. jejuni</td>
<td>HipO</td>
<td>Forward</td>
<td>AATGCAAAAATTTGCTTATATAAACG</td>
<td>123</td>
</tr>
<tr>
<td>C. jejuni</td>
<td>HipO</td>
<td>Reverse</td>
<td>TNCATTTAAATCTTGACTTGGTAAAT</td>
<td></td>
</tr>
<tr>
<td>C. jejuni</td>
<td>HipO</td>
<td>Probe</td>
<td>JOE-ACATACACTACTTATATGCTT-BHQ1</td>
<td></td>
</tr>
<tr>
<td>C. coli</td>
<td>CadF</td>
<td>Forward</td>
<td>GAG AAA TTG TTG TTT TAT GGT TTA GCT GGT</td>
<td>103</td>
</tr>
<tr>
<td>C. coli</td>
<td>CadF</td>
<td>Reverse</td>
<td>ACC TGC TCC ATA ATG GCC AA</td>
<td></td>
</tr>
<tr>
<td>C. coli</td>
<td>CadF</td>
<td>Probe</td>
<td>CY3- CCT CCA CCT TTA TTA TCA AAA GCG CCT TTA GAA A - BHIQ1</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1 Validation diagram of internal DNA extraction protocol

![Figure 1](https://example.com/figure1.png)
Artificial contamination of food matrices

Further validation was also examined using enriched matrices artificially contaminated. Beef, turkey, and broiler chicken minced meat samples were purchased from market, randomly, and were sterilized by freezing and ultraviolet exposure methods. Using a PBS solution, 10-fold serial dilution of C. jejuni and C. coli was performed to obtain the concentrations of 10³, 10⁴, and 10 CFU/mL. Then, in triplicate, 8 mL of Bolton enrichment broth containing 5% defibrinated horse blood and selective supplement (Cefoperazone, vancomycin, trimethoprim, lactate, and amphotericin B) was mixed with 1 g of sterilized minced meat, and 1 mL of each reference strains concentrations (1:10), so as to obtain 100, 10 and 1 CFU/mL as final Campylobacter inouclums concentration in each tube before enrichment. The incubation was achieved under microaerophilic conditions for 44h ± 4h at 41.5 °C. Finally, 1 mL of each enriched broths was subjected to extraction and qPCR as mentioned above (Figure 1).

Optimization of internal protocol

For confirmation of the pure isolates, after culture method, no necessary optimization was needed. Nevertheless, in the artificial contamination of food matrices, three stages in the aforementioned internal DNA extraction protocol were amended and optimized. Firstly, the bacterial pellet was washed three times with PBS solution. Secondly, the lysis step was incubated for additional time at room temperature and lastly, the washing stage was repeated up to three times with ethanol 70%.

Data analysis

Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA, 2007) was used to calculate rate of strains identified by qPCR, the means, and also standard deviation of amplification threshold Cq of each artificially contaminated matrices assay for C. jejuni and C. coli.

Table 2 Main indicators comparison of the three DNA extraction protocols

<table>
<thead>
<tr>
<th>Internal protocol</th>
<th>Wizard® Genomic DNA Purification Kit</th>
<th>PureLink™ Genomic DNA Mini Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost</td>
<td>Cheap</td>
<td>Inexpensive</td>
</tr>
<tr>
<td>Time</td>
<td>1h:30 min</td>
<td>2h</td>
</tr>
<tr>
<td>Efficiency</td>
<td>Similar</td>
<td>1h:35min</td>
</tr>
</tbody>
</table>

The comparison of handling steps of the three DNA extraction methods was closely similar between the three protocols. However, the internal protocol was clearly yielded more advantages than the kits examined in this work. Ruiz-Fuentes et al. (2015) were compared four DNA extraction methods for the detection of Mycobacterium leprae from Ziehl–Neelsen-stained microscopic slides and concluded that the DNA concentration from the Wizard® Genomic DNA Purification Kit was the lowest (1.00 ± 0.18 ng/µL, p ≤ 0.052) among Chelex 100 resin procedure, phenol–chloroform–isoamyl alcohol method, and QiAamp DNA Mini Kit tested. Similar results were observed by Assenmacher et al. (2020). At the best of our knowledge, no published research data, other than the information offered by user guide of this kit, was found describing and/or comparing the advantages or gaps of PureLink™ Genomic DNA Mini Kit for Gram negative or positive bacteria. Despite the appropriate information offered by DNA quality control, this tool consumes a significant amount of DNA (<1–4 ng) (Nikolaev et al., 2018).

Validation of internal protocol

Confirmation of Campylobacter species

Between February and June 2018, the forty Campylobacter spp. pure colonies were recovered from food samples (Broiler chicken, turkey, and beef meats.), biochemically confirmed as C. coli and C. jejuni, were further identified by qPCR using the internal DNA extraction protocol. The findings summarized in Table 3 showed that qPCR identified similar Campylobacter species as confirmed by phenotypique techniques, in which 70% of isolated strains (28/40) were C. coli and 30% (12/40) were C. jejuni.

Table 3 Identification of Campylobacter spp. isolated from food samples

<table>
<thead>
<tr>
<th>Strain (N= 40)</th>
<th>Origin</th>
<th>Biochemical confirmation N (%)</th>
<th>qPCR using internal DNA extraction protocol N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. coli</td>
<td>Broiler chickens, turkeys, and beefs meat.</td>
<td>28 (70)</td>
<td>28 (70)</td>
</tr>
<tr>
<td>C. jejuni</td>
<td></td>
<td>12 (30)</td>
<td>12 (30)</td>
</tr>
</tbody>
</table>

The dominant species reported of Campylobacter genus is C. jejuni (Chon et al., 2018). However, additional investigations found that C. coli was more frequently compared to C. jejuni in meat samples (Guirin et al., 2019; Liu et al., 2019). These findings were similar to those indicated in the present study. Indeed, researchers suggested that the difference in the recovery of Campylobacter spp. can be attributed to culture conditions and method used, phenotypic techniques, seasons, or also types and stage of samples treatment (Butzler, 2004; Iannetti et al., 2020). Owing to the inert biochemical profile of this genus, few phenotypic tests were described to differentiate, mainly, between C. jejuni and C. coli such as hippurate hydrolysis. Despite the false negative and/or positive results that occasionally come across, this test remain useful for some research laboratories (Nakari et al., 2008; Adzitey and Corry, 2011).

Today, molecular methods are the leaders of the most relevant information provided in epidemiological and clinical studies, in which come to overcome the inconveniences of culture and phenotypic techniques, and thus speed up the outcomes (Acke et al., 2009). Real time PCR is one of these methods that use the genetic suitcase to identify and distinguish Campylobacter species worldwide. In their comparative study between hippurate hydrolysis and multiplex PCR for differentiating C. coli and C. jejuni, Adzitey and Corry, (2011) have demonstrated that 17 of the 18 strains were in agreement with both methods used. It was also noticed in the present work, that biochemical tests used to identify Campylobacter species and qPCR using HipO and CadF genes for C. jejuni and C. coli respectively, showed similar results and therefore both tests had proportional sensitivity and efficiency.

RESULTS AND DISCUSSION

This work reported the first comparison of the Pure Link™ Genomic DNA Mini Kit and Wizard Genomic DNA Purification Kit with internal genomic DNA extraction protocol for obtaining the DNA from Campylobacter species.

Comparison of the internal protocol with commercial kits

The current study compared the DNA quality control after extraction process in 1% agarose gel electrophoresis, using the internal DNA extraction protocol, Wizard® Genomic DNA Purification Kit, and PureLink™ Genomic DNA Mini Kit for C. coli and C. jejuni in triplicate. The results showed in Figure 2 indicated that the DNA integrity was similar in the three compared protocols for both tested strains, since the intensity of the bands was clearly the same. Indeed, the internal DNA genomic extraction protocol was simplest, much cheaper, and faster (1h: 30 min) among the Wizard® Promega kit, which took 2hours, and the Pure Link® Invitrogen kit that was the most expensive (Table 2). This latter had similar processing time (1h: 35 min) as internal protocol, since the purification step based on the use of centrifuge columns (PureLink® Spin Column) to speed up the extraction protocol.

Figure 2 A 1% agarose gel electrophoresis of C. coli (Fig. 2A) and C. jejuni (Fig. 2B) DNA extracted in triplicates. 1: Internal protocol, 2: Wizard® Genomic DNA Purification Kit, and3: PureLink™ Genomic DNA Mini Kit, 4: Negative control.
C. jejuni, particularly, in turkey (11.67 ± 20.21) and beef (11.33 ± 19.63) minced meat at concentration of 1 CFU/mL. Likewise, it was noticed some other negative amplifications (Ct >36) for the three contaminated matrices at 1 CFU/mL of C. coli. The means of threshold C values in observed beef minced meat were lower than those of poultry samples.

![Table 4: DNA means observed according to C. jejuni and C. coli](https://www.ncbi.nlm.nih.gov/pubmed/articles/PMCID8319090)

<table>
<thead>
<tr>
<th>Mined meat</th>
<th>C. jejuni (CFU/mL)</th>
<th>C. coli (CFU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broiler chicken</td>
<td>16.33 ± 15.57</td>
<td>16.83 ± 1.76</td>
</tr>
<tr>
<td>Turkey</td>
<td>25.4 ± 3.8</td>
<td>29.5 ± 2.28</td>
</tr>
<tr>
<td>Beef</td>
<td>34.67 ± 2.08</td>
<td>37.67 ± 9.58</td>
</tr>
</tbody>
</table>

SD: Standard deviation.

The artificial contamination with serial dilution of reference strains is usually used in qPCR methods. As qPCR sensitivity is rather higher than other detection techniques combined or not with an enrichment step (Ahmed et al., 2013; El Baaboua et al., 2018). In the internal analysis, the quantitative methodology requires enrichment of samples to detect low level of contamination and thus to improve the isolation rates (Bill et al., 2017). That’s why; the use of the enrichment stage, in our study, was for purpose to increase cells numbers, particularly at concentration of 1 CFU/mL, in order to check the detection limits of this protocol. Abdelhai et al. (2016) have studied three rapid methods (physical, boiling, phenol/ethanol and commercial kit) of Gram-positive DNA extraction and concluded that the DNA extraction yields were significantly increased with amount of the initial culture used and thus the quantity of DNA extracted from the same methodologically was associated with our outcomes of threshold amounts, from 100 to 1 CFU/mL of each inoculum. C. jejuni and C. coli are the most prevalent species isolated in human food, in particular from broiler chickens and turkeys meat (Chon et al., 2018; Gahamanyi et al., 2020). The low recovery levels of Campylobacter in beef were explained, in literature, by the fact that these organisms had less favorable conditions in cattle populations than in poultry (Zhao et al., 2001). Besides that, to the detection limits of decimal dilution series of enrichments of pure cultures were found successively lower in C. lari and C. jejuni compared to C. coli, in the same experimental conditions (Mayr et al., 2010). Base on their results, Mayr et al. (2010) were demonstrated that the limits of detection for C. coli and C. jejuni in qPCR assay were from 5 to 10 and 1-5 CFU/250 mL, respectively. As well as, the post enrichment bacterial counts (from 5 to 10 CFU/250 mL) of 48 hours was revealed the recovery of 10^7 to 10^8 CFU/mL (Mayr et al., 2010). Similar detection limits (at 1 CFU/mL) were noticed in some reactions of C. coli and C. jejuni. Other than the aforementioned reasons, the low Ct obtained at 1 CFU/mL in turkey minced meat and ground beef from enriched culture can be also attributed to the sampling and/or counting errors before and during serial dilution (Ben-David and Davidson, 2014).

Campylobacter jejuni genus is the main cause of food borne illnesses that lead to diversity in manifestations and pathogenesis power, depending on species and host susceptibility (Baaboua et al., 2017). In regards to species ability, the infectious dose plays a critical role. Researchers were confirmed that 15% of human were poisoned after they ingested infectious dose between 8 x 10^7 to 1 x 10^8 of C. jejuni (Black et al., 1998). Moreover, other scientists who infected human volunteers by another C. jejuni strain, proved that only 80% of the volunteers developed positive stool culture, while half of them fell sick at doses of 10^6 CFU, approximately (Havelaar et al., 2009). As a consequence, the internal DNA extraction method was able to detect low infectious doses (up to 1 CFU/mL after enrichment) and can be used in human stool and food specimens for all the Gram-negative bacteria.

Optimization of the internal DNA extraction protocol

The internal DNA extraction protocol was amended when extracting DNA from enriched matrix salter the used of Bolton enrichment broth supplemented with defibrinated horse blood. Indeed, the heme compounds, hemoglobin derivative, inactivate reversibly polymerase in qPCR, which must be removed before starting the DNA extraction procedure. This is the reason why, the cell pellet obtained after centrifugation was washed several times with the PBS solution. Akane et al. (1994) have presented an efficient alternative molecule named Bovine Serum Album (BSA) that can be added to the PCR mixture to enhance the amplification reaction in blood specimens. Likewise, to ensure that DNA extracted from Campylobacter strains were cleaned from salts (mainly Na+), additional wash step was carried out to the DNA pellet, twice and more, with ethanol70% at room temperature. The purification was carried out, herein, to eliminate the contaminants that could disrupt the DNA amplification. For example, a higher concentration of EDTA (> 0.5mM) can affect the fluorescence to 46% (Al-Sound and Rådström, 2001) and can be an inhibitory through depleting the divalent cations such as magnesium (Mg^2+), necessary for Taq polymerase functioning (Datta and LiCata, 2003). More than 5mM and 25mM of sodium acetate and NaCl respectively in DNA extracts, affect negatively the qPCR reaction (Watson and Blackwell, 2000). Moreover, concentration procedures of PCR sensitivity, culture recovery, and thus the quantity of DNA extracted from the same protocol provided similar efficiency compared to the two commercial available kits. The confirmation of Campylobacter pure colonies shown that 28 isolates were C. coli and 12 were C. jejuni, similar to phenotypic methods. Furthermore, a positive amplification was also observed in the three contaminated food matrices, after enrichment, at all examined doses. Except some reactions that were negative at 1 CFU/mL of C. jejuni and C. coli. This was explained by the detection limits of both internal protocol and qPCR. Based on our findings, three crucial steps in determining the extraction of DNA quality of this protocol were amended. Hence, the study highlighted the importance of validating simpler, cheaper, and faster DNA extraction protocol, for each laboratory, as part of future risk assessment, control and monitoring programs of Campylobacter frequency required in molecular studies.

Conflicts of interest: The authors declare that they have no competing interests.

Availability of data and materials: The data used in this study are included within the article.

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REFERENCE


