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Detection of *Campylobacter* sp. from Poultry Feces in Ouagadougou, Burkina Faso

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Abstract

Background: *Campylobacter* contamination in poultry and poultry product has been reported worldwide. The present study aims to determine the prevalence of *Campylobacter* in poultry feces using selective enrichment Bolton broth and multiplex PCR. **Method:** Two methods were used in this study: the first was direct plating of poultry feces into mCCDA agar plates. The second, three antibiotics were used at different concentrations to add in Bolton broth supplemented. These antibiotics were Rifampicin (Oxoid, Nepean, Ontario) with 10 mg/L, colistin (Oxoid, Nepean, Ontario) with 1 mg/mL and 2 mg/mL; trimethoprim (Oxoid, Nepean, Ontario) with 10 mg/L. The colonies with typical *campylobacter* morphology on blood agar (little, red and ring colonies) were further identified to the species level by multiplex polymerase chain reaction (PCR). **Results:** The addition of colistin (2 mg/mL) to the Bolton broth with selective supplements enhanced the selective isolation of *Campylobacter* strains. Out of the 52 feces samples, 18 (34.61%) were positive for *campylobacter* and direct plating on mCCDA 11 (21.15%) *campylobacter* strains ($p < 0.05$). The PCR results have shown that 17 (94.45%) of the *campylobacter* strains detected belonged to *Campylobacter coli* and 1 (5.55%) strain to *Campylobacter jejuni*. **Conclusion:** Although it is known to be difficult to isolate *Campylobacter* from animal feces samples, this study shows that antibiotic selective pressure improves the isolation efficiency of *Campylobacter* from poultry feces.

Keywords

Campylobacter, Poultry Feces, Selective Method, Multiplex PCR

1. Introduction

Campylobacter is a leading cause of human gastroenteritis worldwide [1]. Poultry is considered to be the major reservoirs of *Campylobacter*, and unhygienic handling or consumption of raw or undercooked poultry products is the main cause of *Campylobacter* infections [2] [3]. *Campylobacter* strains detected in feces from slaughtered poultry can contaminate poultry carcasses if hygienic practices failed during the gastrointestinal removing process [4] [5]. In the last decade, *Campylobacter* isolated from human or animal samples is showing more and more resistance to a variety of antibiotics [6]. However, the epidemiology of *Campylobacter* is still not elucidated in many countries. In Burkina Faso, there is a lack of sufficient resources to routinely employ modern methods of clinical and molecular microbiology for screening, prevention and policy-making purposes related to the gastroenteric disease. Conventional biochemical-based assays for the identification of *Campylobacter* species are time-consuming and laborious due to the fastidious growth requirements of these species and the paucity of informative biochemical characteristics [7]. Moreover, *Campylobacter* strains identification requested selective protocol, which is relatively expensive for developing countries. It still remains a high research priority to improve the strategies for management as well as an improved method for the isolation and identification of these pathogens. Molecular methods, especially Polymerase chain reaction (PCR)-based species identification for *Campylobacter* spp. provide more reliable identification of thermophilic *Campylobacters*, as they enhance the sensitivity and specificity of the detection process [8]. In Burkina Faso, very few studies have been undertaken to determine the prevalence of *Campylobacter* in humans and/or poultry [9] [10]. To better understand the epidemiology of *campylobacter*iosis in Burkina Faso, this study aims to determine the prevalence of *Campylobacter* in poultry feces using an optimized identification protocol and multiplex PCR.

2. Materials and Methods

Sampling

From July to August 2017, fecal samples from slaughtered chickens (n = 54) were collected from the local poultry sellers in two retail markets of Ouagadougou, Burkina Faso. There were no records available concerning poultry farms, but according to the poultry sellers, the chickens originated from many farms across the country. Immediately after the poultry was slaughtered, the whole intestine was collected after evisceration, placed in sterile plastic bags, and transported in a cool box (4˚C) to the laboratory. The cecal content of poultry was put in a sterile cryotube and kept at −20˚C for future *Campylobacter* isolation. The cryotubes were sent to the laboratory "Chaire de Recherche en Salubrité des Viandes (CRSV)" at the faculty of veterinary medicine /the University of Montréal for isolation and identification.

***Campylobacter* isolation and identification**

Two methods were used for *Campylobacter* isolation in CRSV:

A loopful of poultry cecal content was streaked directly onto modified charcoal cefoperazone desoxycholate agar (mCCDA) (Oxoid, Nepean, Ontario) and incubated at 42˚C for 48 h in a microaerobic atmosphere using Oxoid's microaerobic atmosphere generation system (Oxoid).

In parallel, 1 g of caecal content was inoculated into 9 ml of Bolton broth (Oxoid, Nepean, Ontario) supplemented with selective supplements (SR0155 E, Oxoid, Nepean, Ontario). Other antibiotics were tested as an additive to limit the overgrowth of cephalosporin-resistant enterobacteria. Three different antibiotics with different concentrations were used to make a serial dilution using Bolton broth (10^{-1} to 10^{-7}) by inoculating the reference strain of Camylobacter (ATCC 33291) and samples. These antibiotics were: rifampicin (Oxoid, Nepean, Ontario) with 10 mg/L, colistin (Oxoid, Nepean, Ontario) with 1 mg/mL or 2 mg/mL as well as trimethoprim (Oxoid, Nepean, Ontario) with 10 mg/L. The retained antibiotic for the modified Bolton broth was colistin 2 mg/mL because this antibiotic was more selective for *Campylobacter* compare to the other tested antibiotics.

Then, for the samples processing, 1 g of poultry cecal content was added to 9 ml of modified Bolton broth, mixed and incubated at 42˚C for 48 h in a microaerobic atmosphere. For the control strain (*C. jejuni* strain ATCC 33291), one colony from the blood Agar plate was added to 9 ml of modified Bolton broth, mixed and incubated at 42˚C for 48 h in a microaerobic atmosphere. After enrichment, a loopful of the modified Bolton broth was streaked onto modified charcoal cefoperazone desoxycholate agar (mCCDA) (Oxoid) and incubated at 42˚C for 48 h in a microaerobic atmosphere.

For samples and control strains, after incubation, the typical looking *Campylobacter* colonies were purified on mCCDA (Oxoid) agar plates and incubated at 42˚C for 48 h in a microaerobic atmosphere. Then, the pure colonies were streaked onto blood agar (Oxoid) and incubated at 42˚C for 48 h in a microaerobic atmosphere. The colonies with typical *Campylobacter* morphology on blood agar were kept and further identified down to the species level by polymerase chain reaction (PCR).

The presence and identification of *Campylobacter* strains were detected by multiplex PCR. The primers and PCR conditions were as previously described [7], with slight modification. Briefly, for DNA extraction, a single loopful of culture was inoculated in 50 µl NaOH (25 mM) in a 1.5 ml microcentrifuge tube using a disposable loop (1 mm diameter), and the cell mixture was heated at 100˚C for 10 min. After neutralization with 50 ml tris/HCl buffer (80 mM, pH 7.5), cell debris was pelleted by centrifugation at 20,000 g, 4˚C, for 5 min, and the supernatant was used as template DNA. The final multiplex PCR comprised: 1 ml template DNA; 0.2 mM primers C412F, C1228R, C-1, C-3, CC18F, CC519R, CU61F, CU146R, MG3F, CF359R, CLF, CLR, HYO1F and HYOFET23SR; and 0.56Q solution (Qiagen, Montréal, Canada) in 16 multiplex PCR master mix

(Qiagen, Montréal,Canada). The final volume per well was adjusted to 25 µl with sterile water. Primers sequences were previously described by Yamazaki-Matsune *et al.* [7].

The cycling conditions used were 95˚C for 15 min, 25 cycles each of 95˚C for 0.5 min, 58˚C for 1.5 min and 72˚C for 1 min and a final extension at 72˚C for 7 min. Samples were held at 4˚C prior to analysis. PCR amplicons were visualized on a 1% agarose (Thermo, Fisher Scientific, Ottawa, Canada) gel stained with Sybrsafe (Thermo, Fisher Scientific, Ottawa, Canada). The positive control consisted of DNA extracted from *C. jejuni* strain ATCC 33291 while the negative control contained no DNA.

3. Results

Optimization of the isolation method

The results show that direct plating of poultry feces on mCCDA plates was not selective like enrichment in Bolton broth supplemented with additional antibiotics. The enrichment method using Bolton broth supplemented with additional colistin (2 mg/mL) has given good results in selecting the *campylobacter* strains and inhibiting the other flora. This selective broth yielded significantly better isolation rate of *Campylobacter* than those achieved on using normal Bolton broth supplemented without additional antibiotic or direct plating. Enrichment in selective broth allowed the detection of 18 (34.61%) *Campylobacter* strains from 52 feces samples and direct plating on mCCDA 11 (21.15%) *Campylobacter* strains ($p < 0.05$).

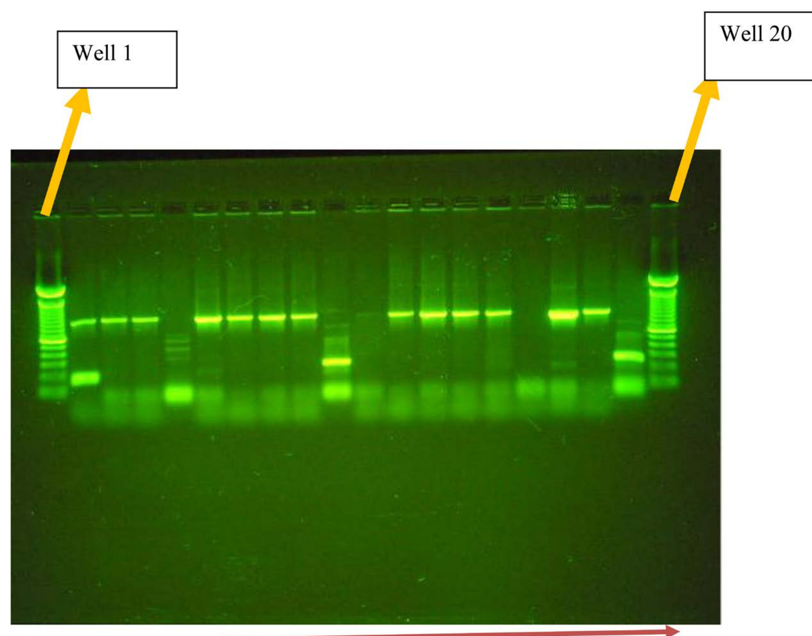
Prevalence of *Campylobacter*

A multiplex PCR assay has been used for the identification of six common *Campylobacter* species, namely *Campylobacter coli*, *Campylobacter fetus*, *Campylobacter hyointestinalis subsp. hyointestinalis*, *Campylobacter jejuni*, *Campylobacter lari* and *Campylobacter upsaliensis*. Figure 1 shows picture of a typical electrophoretogram from PCR. Out of the 52 feces samples, 18 (34.61%) were positive for *Campylobacter* genus.

The PCR results have shown that 17 (94.45%) of the *Campylobacter* strains detected belonged to *Campylobacter coli* and 1 (5.55%) strains belonged to *Campylobacter jejuni*.

4. Discussion

Poultry is an important reservoir of *Campylobacter* and chicken and poultry product has been involved in *campylobacter*iosis worldwide. In this study, we investigated using optimization protocol and multiplex PCR to detect *Campylobacter* presence in poultry feces. The result shows that the enrichment method using Bolton broth supplemented with additional colistin at 2 mg/mL yielded significantly better isolation rate and selectivity than those achieved on using normal Bolton broth supplemented without additional antibiotic or direct plating on mCCDA. From the direct plating, the inconvenience is the growth of



Legend: Well 1 and 20 = Ladder; Well 2 (−) Reference strain (*C. jejuni*); Well 3 (+, *Campylobacter sp*); Well 4 (+, *Campylobacter sp*); Well 5 (−); Well 6 (+, *Campylobacter sp*); Well 7 (+, *Campylobacter sp*); Well 8 (+, *Campylobacter sp*); Well 9 (+, *Campylobacter sp*); Well 10 (−); Well 11 (+, *Campylobacter sp*); Well 12 (+, *Campylobacter sp*); Well 13 (+, *Campylobacter sp*); Well 14 (+, *Campylobacter sp*); Well 15 (+, *Campylobacter sp*); Well 16 (−); Well 17 (+, *Campylobacter sp*); Well 18 (+, *Campylobacter sp*); Well 19 (−).

Figure 1. Typical electrophoretogram.

competitive Gram-negative bacteria, which influence the presence of *Campylobacter*. Previous studies have reported that increased selective pressure in selective agar and/or enrichment broth enhances *Campylobacter* isolation from various samples. Kim *et al.*, [11] reported that the addition of polymyxin B, rifampicin, or both to the Bolton selective supplements enhanced the selective isolation of *Campylobacter* isolation from wastewater. Yoo *et al.* [12] reported that the addition of rifampicin (10 µg/ml) or polymyxin B (5 IU/ml) to Bolton agar (Bolton agar with Bolton supplement) restrained the growth of non-*Campylobacter* without any inhibition of *C. jejuni* and *C. coli* in fresh produce foods. Chon *et al.* [13] demonstrated that the addition of high concentrations of polymyxin B to the Bolton supplement in enrichment procedure improved the efficiency of *C. jejuni* and *C. coli* recovery and suppressed background competing bacteria. Consistently, our results showed that the supplementation with additional colistin (2 mg/mL) improved the efficacy of *Campylobacter* isolation from poultry feces.

In this study, the result revealed a low prevalence (34.61%) of *Campylobacter* species in poultry feces, with a high predominance of *C. coli*. This finding could be explained by the fact that samples were frozen at −20˚C before to send to the laboratory "Chaire de Recherche en Salubrité des Viande (CRSV)", department of veterinary medicine, University of Montréal. The result highlights a good hypothesis that *C. coli* could be more resistant to the environmental condition than

C. jejuni, which could be tested in future studies. However, many authors demonstrated that if the birds are older, there is more *C. coli* because *C. jejuni* would be gradually replaced by *C. coli* [14] [15]. Contrast results were reported in our previous study where, highest prevalence (67.96%) of *Campylobacter* in poultry, with a high predominance of *C. jejuni* [10]. The lowest prevalence of *campylobacter* from poultry (15.52%) feces compare to our present result has been reported by Rawat *et al.* [8] in India. In contrast, Osbjer *et al.*, [16] reported 56% prevalence of *Campylobacter* in chicken feces in rural Cambodia. The difference in *Campylobacter* prevalence in poultry feces could be explained by the difference in isolation methods, the sampling conditions, geographical areas, and farming conditions.

The *Campylobacter* species found in this study were *C. coli* and C. jejuni, which have been reported worldwide as the most frequently associated with human infection [10] [17]. Moreover, Whiley *et al.* [18] reported in their study that the consumption of contaminated poultry is the primary cause of developing human *campylobacter*iosis.

5. Conclusion

These findings show that more attention is needed during poultry carcasses processing plan to avoid carcasses contamination, and hygienic practices should be respected during carcasses preparation to avoid the risk of cross-contamination. This study highlights also the need for developed countries to invest more resources into *Campylobacter* research to identify possible sources of campylobacteriosis and therefore put in place adequate measures to lower the impact of the foodborne pathogens on public health.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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