Differential Survival of Hyper-Aerotolerant Campylobacter jejuni under Different Gas Conditions

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Campylobacter jejuni accounts for a significant number of foodborne illnesses around the world. C. jejuni is microaerophilic and typically does not survive efficiently in oxygen-rich conditions. We recently reported that hyper-aerotolerant (HAT) C. jejuni are highly prevalent in retail poultry meat. To assess the capabilities of HAT C. jejuni in foodborne transmission and infection, in this study, we investigated the prevalence of virulence genes in HAT C. jejuni and the survival in poultry meat in atmosphere at a refrigeration temperature. When we examined the prevalence of eight virulence genes in 70 C. jejuni strains from raw poultry meat, interestingly, the frequencies of detecting virulence genes were significantly higher in HAT C. jejuni strains than aerosensitive C. jejuni strains. This suggests that HAT C. jejuni would potentially be more pathogenic than aerosensitive C. jejuni. Under aerobic conditions, aerosensitive C. jejuni survived at 4°C in raw poultry meat for 3 days, whereas HAT C. jejuni survived in poultry meat for a substantially extended time; there was a five-log CFU reduction over 2 weeks. In addition, we measured the effect of other gas conditions, including N₂ and CO₂, on the viability of HAT C. jejuni in comparison with aerosensitive and aerotolerant strains. N₂ marginally affected the viability of C. jejuni. However, CO₂ significantly reduced the viability of C. jejuni both in culture media and poultry meat. Based on the results, modified atmosphere packaging using CO₂ may help us to control poultry contamination with HAT C. jejuni.

Keywords: Campylobacter, aerotolerance, virulence genes, bacterial survival, pathogen inhibition

INTRODUCTION

Campylobacter is a leading bacterial cause of human gastroenteritis, annually accounting for approximately 166 million diarrheal cases around the world, particularly in developed countries (Kirk et al., 2015). Campylobacter infection in humans develop fever, vomiting, abdominal pains, and diarrhea, and in some cases Guillain–Barré syndrome, an autoimmune disorder characterized by acute and progressive neuromuscular paralysis (Young et al., 2007). Human infection with C. jejuni is facilitated by the function of various virulence factors involved in toxin production (e.g., cdtABC), cell adhesion (e.g., cadF, peb1A, and pldA) and invasion (e.g., ciaB), and colonization of gastrointestinal tracts (Bolton, 2015).
Campylobacter is isolated from a wide range of domestic animals and wildlife (Jokinen et al., 2011). In particular, the gastrointestinal tracts of poultry are colonized by Campylobacter jejuni, the major human pathogenic species of Campylobacter, at the level of 10⁶–10⁸ CFU/g feces or higher (Hermans et al., 2011). Poultry meat is often contaminated with C. jejuni during poultry processing, and human campylobacteriosis is most frequently associated with the consumption of contaminated poultry products (Skarp et al., 2016). In addition, cross-contamination in the kitchen is also an important risk factor transferring Campylobacter (Chai et al., 2008; Luber, 2009). It has been estimated that a two-log reduction in the number of Campylobacter on chicken carcasses may lead to approximately a 30-fold reduction in the number of human campylobacteriosis cases (Rosenquist et al., 2003). To control Campylobacter contamination of poultry, various intervention strategies have been examined at the pre- and post-harvest levels, such as bacteriocin and bacteriophages (Hermans et al., 2011; Umaraw et al., 2017).

Unlike other enteric pathogenic bacteria, C. jejuni exhibits unique microbiological features. For example, C. jejuni is asaccharolytic and has limitations in the utilization of hexose sugars, including glucose, because of the lack of 6-phosphofructokinase in the glycolysis pathway (Parkhill et al., 2000; Velayudhan and Kelly, 2002). To supply carbon sources, C. jejuni relies on the utilization of amino acids, organic acids (e.g., lactic acid), and fucose in some strains (Leach et al., 1997; Thomas et al., 2011; Stahl et al., 2012). In addition, C. jejuni is microaerophilic and capnophilic and requires both O₂ and CO₂ for growth preferably at 5–10% and 1–10%, respectively (Bolton and Coates, 1983). Despite the fastidious nature of Campylobacter, it has not been understood how Campylobacter causes such a significant number of human infection cases around the world.

Various tolerance mechanisms have been reported to support the survival of Campylobacter under harsh stress conditions, such as heat, cold, acid, and desiccation stresses (Murphy et al., 2006). In addition, Campylobacter produces biofilms and switches its physiological state to a viable but nonculturable (VBNC) cell to promote survival under stress conditions. In C. jejuni, biofilm formation is stimulated under aerobic conditions, and aeration triggers the formation of VBNC cells (Oh et al., 2015b, 2016), suggesting C. jejuni is equipped with multiple survival mechanisms that may support the viability of C. jejuni under oxygen-rich conditions. Besides these survival mechanisms, aerotolerance would be the front-line survival mechanism of C. jejuni when this microaerophilic pathogen encounters the aerobic environment (Bronowski et al., 2014). Despite our perception about oxygen-sensitivity in C. jejuni, interestingly, we recently reported that hyper-aerotolerant (HAT) strains of C. jejuni are highly prevalent in retail poultry meat; the HAT strains survive longer than 24 h in vigorous aerobic shaking at 200 rpm. Also, HAT C. jejuni often belongs to the multilocus sequence typing (MLST) clonal complexes (CCs) that are frequently implicated in human infection (Oh et al., 2015a), suggesting that HAT C. jejuni might be closely related to human infection. To evaluate the virulence potential of HAT C. jejuni, in this study, we investigated the prevalence of virulence genes in HAT C. jejuni strains. In addition, we measured the survival of HAT C. jejuni under different gas conditions, such as N₂ and CO₂, aiming to develop intervention strategies to control HAT C. jejuni in poultry meat by using modified atmosphere packaging (MAP) with different gases, since aerotolerance confers tolerance to oxygen, not other gases.

**MATERIALS AND METHODS**

**Bacterial Strains and Culture Conditions**

Seventy C. jejuni strains that were isolated from poultry were used in this study (Oh et al., 2015a). C. jejuni NCTC 11168 is the first genome-sequenced strain of Campylobacter and was used as a control in the study (Parkhill et al., 2000). C. jejuni 81–176 was used as a positive control for PCR detection of virB11 (Bacon et al., 2002). In our previous study, we first reported high prevalence of HAT C. jejuni that can effectively survive in a vigorous aerobic condition, such as aerobic shaking at 200 rpm (Oh et al., 2015a). Based on the level of aerotolerance, we arbitrarily divided C. jejuni into three different groups: (1) aerotolerant C. jejuni that loses viability before 12 h by aerobic shaking at 200 rpm, (2) aerotolerant C. jejuni that loses viability between 12–24 h by aerobic shaking at 200 rpm, and (3) HAT C. jejuni that survives even after 24 h of aerobic shaking at 200 rpm (Oh et al., 2015a). The 70 C. jejuni poultry strains were isolated from retail poultry meat in our previous study and consisted of 20 aerotolerant strains, 25 aerotolerant strains, and 25 HAT strains (Oh et al., 2015a). The C. jejuni strains were routinely grown on Mueller–Hinton (MH) agar plates (Difco) at 42°C under microaerobic conditions (85% N₂, 5% O₂ and 10% CO₂).

**Determination of C. jejuni Survival under Different Gas Conditions**

Campylobacter jejuni survival was determined in MH media and chicken meat at 4°C in normal atmospheric conditions and under CO₂ and N₂. Frozen C. jejuni strains in 10% glycerol were inoculated on MH agar plates and incubated at 42°C under microaerobic condition. Overnight cultures of strains of C. jejuni grown on MH agar plates were harvested with fresh MH broth and diluted in MH broth to an optical density at 600 nm (OD600) of 0.1. The bacterial suspension was transferred to multiple 96-well plates, and the 96-well plates were incubated at 4°C in air and in an anaerobic jar filled with either CO₂ or N₂. In addition, N₂ gas condition was constructed with 100% nitrogen gas flushing and CO₂ condition was generated with gas pack (>97% CO₂). To prevent desiccation, a container with water was placed nearby the 96-well plates in a refrigerator. Samples were taken at predetermined time for enumeration. In addition, the survival of two strains of C. jejuni, which were randomly chosen from each aerotolerance group [HAT strains (#12 and #21), aerotolerant strains (#4 and #29), and aerotolerant strains (#24 and #66)], was determined in raw chicken meat; these strains were selected from different treatments.
MLST CCs based on their aerotolerance level. Approximately one-gram of raw chicken meat, including skin and muscle, was prepared with a sterilized razor and placed in a 12-well plate. After applying an aliquot (100 µl) of C. jejuni suspension (approximately 8 × 10^8 CFU/ml) onto each portion of meat and skin mixture, the plate was stored at 4°C under three different gas conditions, including normal atmosphere, CO₂, and N₂. Due to the potential indigenous C. jejuni in poultry meat, controls were prepared without addition of C. jejuni. The poultry meat samples were transferred to a 50 ml tube containing 2 ml of fresh MH broth. After vortexing for 2 min, the supernatant was collected, serially diluted, and spread onto MH agar plates for enumeration. Each experiment was carried out with duplicate samples, and the experiment was repeated three times.

**PCR Detection of Virulence Genes**

Overnight cultures on MH agar at 42°C under microaerobic conditions of C. jejuni strains were collected in PBS (pH 7.2). Bacterial suspension of overnight culture of C. jejuni strains were diluted in PBS to an OD600 of 0.01 (approximately, 8 × 10^6 CFU/ml) and boiled for 10 min to release gDNA. After centrifugation, the supernatant was used as a template. To evaluate the potential virulence of HAT C. jejuni strains, we investigated the prevalence of eight important virulence genes (cadF, cdtB, ciaB, docA, iam, peb1A, pldA, and virB11), which are associated with toxin production, cell adhesion and invasion, and colonization of gastrointestinal tracts in chickens with PCR according to Oh et al. (2013) and Konkel et al. (1999a). Each experiment was carried out with duplicate samples, and the experiment was repeated three times. For each experiment, the bacterial titre was amplified from C. jejuni NCTC11168, and virB11 was amplified from C. jejuni 81–176. The PCR mixture was amplified with the following temperature (NCTC11168, and C. jejuni cadF in Table 1 with ExTaq polymerase (Takara, Japan). Primers used are listed in Table 1. The positive controls for six virulence genes, such as cadF, cdtB, ciaB, docA, iam, peb1A, and pldA, were amplified from C. jejuni NCTC11168, and virB11 was amplified from C. jejuni 81–176. The PCR mixture was amplified with the following conditions: initial denaturation at 96°C for 3 min followed by 35 cycles of denaturation 96°C for 30 s, variable annealing temperature (cdtB, ciaB, cadF and pldA at 45°C, docA, peb1 and virB11 at 50°C, iam at 53°C) for 30 s, extension at 72°C for 1 min 20 s and the final extension at 72°C for 7 min. The results were analyzed by electrophoresis with 1% agarose gels and SYBR safe staining dye (Invitrogen).

**Statistical Analysis**

Two-way ANOVA was performed by using GraphPad Prism 6 (GraphPad Software Inc., United States). Chi-square distribution was used to analyze if the prevalence of virulence genes is dependent on aerotolerance by using SPSS Statistics 21.0 (IBM Predictive Software, United States).

**RESULTS**

**Effect of Aerotolerance on C. jejuni Survival in Chicken Meat**

To evaluate the impact of hyper-aerotolerance on the survival of C. jejuni in poultry meat in this study, raw poultry meat was spiked with two strains of C. jejuni from each aerotolerance group (i.e., aerosensitive, aerotolerant, and HAT C. jejuni groups) and incubated at 4°C under aerobic conditions. The aerosensitive C. jejuni strains lost their viability on poultry meat within 3 days, and the aerotolerant C. jejuni strains survived for 7 days (Figure 1). Interestingly, HAT C. jejuni strains survived in poultry meat for 2 weeks (Figure 1). This means that HAT C. jejuni strains survived in food in atmospheric conditions approximately four times longer than aerosensitive strains of C. jejuni. The results showed that aerotolerance significantly affects the viability of C. jejuni in poultry meat under aerobic conditions.

**Prevalence of Virulence Genes in HAT C. jejuni Strains**

In 70 strains of C. jejuni from poultry meat, the frequencies of detecting virulence genes were 100, 97.1, 68.6, 81.4, 57.1, 84.3, 64.3, and 11.4% for cadF, cdtB, ciaB, docA, iam, peb1, pldA, and

**Table 1** Primers used in this study.

| Gene  | Primer  | Sequence (5’-3’) | Size (bp) | Reference         |
|-------|---------|------------------|-----------|-------------------|
| cadF  | cadF_F  | TTGAAGGTAAATTAAGATATG | 400       | Konkel et al., 1999a |
|       | cadF_R  | CTAATACCTAAAGTTGAAGC  |           |                   |
| cdtB  | cdtB_F  | GTTAAAATCCCTGCTATCAACCA | 495       | Bang et al., 2001  |
|       | cdtB_R  | GTCGACCTTGGGAATTTGCAAGGC | 1163      | Konkel et al., 1999a |
| ciaB  | ciaB_F  | GTCGAAGTGCGAGCT  | 725       | Muller et al., 2006 |
|       | ciaB_R  | GCTCTTAAATTTACTGATGCG  |           |                   |
| docA  | docA_F  | ATAGGCTGCGGTTTGCG  | 518       | Konkel et al., 1999a |
|       | docA_R  | GTCTTGGCAAGTGATATG  |           |                   |
| iam   | iam_A_F  | GCACAAAATATATACATACAA | 775       | Biswas et al., 2011 |
|       | iam_A_R  | TTAACGAGTACTATGAAGG  |           |                   |
| peb1  | peb1_F  | TATAAGCTACTAGTGAGG  | 913       | Datta et al., 2003  |
|       | peb1_R  | TTTTCGACTTGGCTATG  |           |                   |
| pldA  | pldA_F  | AAGCTTATGGCGTTT   | 708       | Bacon et al., 2002  |
|       | pldA_R  | TTACGCTATGCGCTATG  |           |                   |
| virB11| virB11_F | GAACAGGAAAGTGAAAAACTAGC | 301       |                   |
|       | virB11_R | TTCCGGATTTGGCGATATG |           |                   |
log CFU reduction in HAT strains of C. jejuni, a three log CFU reduction in aerotolerant strains, and a two log CFU reduction in HAT strains were similar between days 3 and 7 (Figure 3). Meat was detected in day 14 (data not shown).

Figure 3 | Incubation in N₂ broth under the normal atmospheric conditions within 3 days. The major gases used for MAP are N₂, CO₂, and O₂. Thus, we selected N₂ and CO₂ for the viability testing of HAT C. jejuni strains. Consistent with their aerotolerance level, there was about approximately a four log reduction in CFU in aerotolerant strains of C. jejuni, a three log CFU reduction in aerotolerant strains, and a two log CFU reduction in HAT strains of C. jejuni at 4°C in MH broth under the normal atmospheric conditions within 3 days. Incubation in N₂ reduced the survival of C. jejuni, and CO₂ further decreased CFU counts in HAT C. jejuni, compared with the aerobic conditions (Figure 3). The CFU reduction in all the strains were similar between days 3 and 7 (Figure 3), and no C. jejuni was detected in day 14 (data not shown).

**Viability of HAT C. jejuni Strains in Different Gas Atmospheres**

The survival of HAT C. jejuni measured under different gaseous conditions. In the food industry, MAP is often employed to extend the microbial shelf-life of meat, and O₂, N₂, and CO₂ are the major gases used for MAP. Thus, we selected N₂ and CO₂ for the viability testing of HAT C. jejuni strains (Table 2), suggesting that HAT C. jejuni would potentially be more pathogenic to humans than aerotolerant C. jejuni.

**Impact of Different Gas Atmosphere on the Survival of HAT C. jejuni in Poultry Meat**

The viability of C. jejuni strains belonging to different aerotolerance groups was determined in poultry meat stored in different gas atmospheres. In N₂, aerotolerant and HAT C. jejuni strains were detected for 14 days, whereas aerosensitive strains survived for 7 days (Figure 4A). Compared to aerobic conditions (Figure 1), N₂ did not reduce the viability of HAT C. jejuni strains in poultry meat. In CO₂, however, HAT strains of C. jejuni survived only for a week (Figure 4B); this is a significant viability reduction compared to atmospheric conditions where HAT C. jejuni strains survived for 2 weeks in poultry meat (Figure 1). The results exhibit that HAT C. jejuni did not survive well in CO₂, compared to aerobic conditions.

**DISCUSSION**

Despite the well-known microaerophilic characteristic of C. jejuni, our previous study showed that some C. jejuni strains are highly tolerant to aerobic stress and these strains are highly prevalent in poultry meat (Oh et al., 2015a). In addition, Rodrigues et al. (2015) recently characterized an unique human isolate of C. jejuni strain, named Bf, which can grow aerobically, suggesting that some C. jejuni strains are highly resistant to aerobic stress. Increased tolerance to aerobic stress would enable C. jejuni to survive during transmission to humans through foods. This would significantly impact the safety of poultry meat because of frequent contamination of poultry meat by Campylobacter. In this study, we demonstrated that HAT C. jejuni survived in raw poultry meat at 4°C significantly longer than aerosensitive C. jejuni (Figure 1), confirming the potential threat of HAT C. jejuni on the safety of fresh poultry meat.

The cadF and cdt genes are detected in C. jejuni strains from poultry at high frequencies (Rozyn et al., 2005). Similarly, in this study, cadF and cdt genes were detected in all and most (97.1%) C. jejuni strains, respectively (Table 2). The iam locus has been detected in C. jejuni chicken isolates at 54.7% (Rozyn et al., 2005). The pldA andciaB genes have been detected from C. jejuni poultry isolates at the frequencies of 63.6 and 67.3%, respectively (Melo et al., 2013). Hanning et al. (2010) reported relatively low detection frequencies of ciaB (40%) and pldA (56%) in C. jejuni isolates from poultry carcasses. The virB11 gene is located in the virulence plasmid pVir, which is often detected in C. jejuni strains that cause bloody diarrhea (Bacon et al., 2002; Tracz et al., 2005). The prevalence of virB11 was 10.7~17% in human clinical isolates and 9.5~14% in poultry isolates (Datta et al., 2003; Tracz et al., 2005). When the results were sorted based on the aerotolerance level, the frequencies of detecting virulence genes were significantly higher in HAT C. jejuni strains in comparison with aerosensitive C. jejuni strains (Figure 2 and Table 2). Interestingly, the most substantial differences in the frequency of detection were observed in the genes associated with invasion, including ciaB and iam (Figure 2 and Table 2). CiaB shares similarities with SipB (Salmonella invasion protein B) from Salmonella and IpaB (invasion plasmid antigen B) from Shigella flexneri and is translocated to human epithelial cells. Even though a knockout mutation of ciaB does not affect C. jejuni adhesion to INT407 cells, it significantly

**virB11**, respectively (Figure 2 and Table 2). When we clustered the results based on the aerotolerance level, interestingly, the detection frequencies in HAT C. jejuni strains were higher than those in aerosensitive C. jejuni strains (Table 2), suggesting that HAT C. jejuni would potentially be more pathogenic to humans than aerotolerant C. jejuni.

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**Figure 1** | Survival of aerosensitive, aerotolerant, and HAT C. jejuni strains in poultry meat at 4°C under aerobic conditions. Two C. jejuni strains were randomly selected from each aerotolerance group and used to spike raw poultry meat in duplicate. The results indicate the means and standard deviations of duplicate samples of the two different strains in a single experiment. Three independent experiments were performed, and similar results were obtained in all the experiments. The statistical analysis was performed with two-way ANOVA in comparison with aerosensitive strains. *P ≤ 0.05, **P ≤ 0.01.
Oh et al. Aerotolerance in Campylobacter

FIGURE 2 | Detection of virulence genes in 70 strains of C. jejuni from poultry meat. The results show the prevalence of eight virulence genes in hyper-aerotolerant (A), aerotolerant (B), and aerosensitive (C) strains of C. jejuni. Positive controls (D) were amplified from C. jejuni NCTC11168 (cadF, cdtB, ciaB, docA, iam, peb1 and pldA) and 81–176 (virB11). Controls were included each batch of PCR testing, and representative results were presented.

TABLE 2 | Prevalence (%) of virulence genes in 70 isolates of C. jejuni from poultry meat.

|                | cadFND | cdtBNS | ciaB**** | docA** | iam**** | peb1* | pldA**** | virB11NS |
|----------------|---------|---------|-----------|--------|---------|-------|----------|---------|
| HAT C. jejuni (n = 25) | 100     | 100     | 100       | 100    | 100     | 100   | 96       | 20      |
| Aerotolerant C. jejuni (n = 25) | 100     | 96      | 52        | 68     | 20      | 84    | 48       | 8       |
| Aerosensitive C. jejuni (n = 20) | 100     | 95      | 50        | 75     | 50      | 65    | 45       | 5       |
| Total (n = 70) | 100     | 97.1    | 68.6      | 81.4   | 57.1    | 84.3  | 64.3     | 11.4    |

Statistical significance was performed by chi-square distribution with SPSS ver.21 (IBM). *P ≤ 0.05, **P ≤ 0.01, ****P ≤ 0.0001, NS, Not Significant; ND, Not determined.

impairs the internalization of C. jejuni into INT407 cells (Konkel et al., 1999b). The invasion-associated marker (iam) locus was first reported by Carvalho et al. (2001) with random amplified polymorphic DNA techniques (RAPD) and was detected in 85% of invasive strains and 20% of non-invasive strains. The detection frequencies of pldA were also significantly different between HAT and aerosensitive C. jejuni strains (Figure 2 and Table 2). The pldA gene encodes an outer membrane phospholipase A that is involved in hemolysis (Grant et al., 1997). The pldA and ciaB genes also play a role in C. jejuni colonization of chicken intestines (Ziprin et al., 2001). The increased prevalence of the virulence genes in HAT C. jejuni strains suggests that HAT C. jejuni would be more pathogenic to humans than aerosensitive C. jejuni.

The transmission of C. jejuni to humans is primarily mediated by contaminated food, mainly poultry meat. Due to the fastidiousness and oxygen sensitivity, C. jejuni is not expected to survive efficiently during foodborne transmission in oxygen-rich, atmospheric conditions. However, our results indicate that HAT C. jejuni survives longer in poultry meat than aerosensitive strains during transmission to humans in air and would be more capable of causing human infection (Figure 1). In this study, we observed that the survival of HAT C. jejuni is significantly reduced under CO2 (Figures 3, 4).
FIGURE 3 | Survival of aerosensitive (A), aerotolerant (B), and HAT (C) strains of C. jejuni in MH broth under different gas conditions. Incubation was carried out in atmospheric, N₂, and CO₂ conditions. Two strains from each aerotolerance group were randomly selected, and each strain was inoculated in MH broth in triplicate. The initial CFU was adjusted to be approximately 10⁸ CFU/ml for all the samples and is indicated with blue dashed lines. The results show the mean and standard deviation of the triplicate samples of two different strains in a single experiment. The experiment was repeated three times, and similar results were obtained in the three independent experiments. Two-way ANOVA testing was carried out for statistical analysis. **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001.

FIGURE 4 | Survival of aerosensitive, aerotolerant, and HAT C. jejuni strains at 4°C in poultry meat in N₂ (A) and CO₂ (B). Two strains from each aerotolerance group were randomly selected for the experiment. The results indicate the means and standard deviations of duplicate samples of the two different strains in a single experiment. Three independent experiments were performed, and similar results were obtained all the experiments. The statistical analysis was carried out with two-way ANOVA. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001.

This provides important scientific background for developing methods to control HAT C. jejuni with MAP. In food industry, CO₂, N₂ and their combinations are generally used for the development of MAP of foods. Compared to aerobic conditions, the survival of HAT C. jejuni strains in raw poultry meat was significantly reduced by CO₂ (Figures 1, 4B). Meredith et al. (2014) tested different compositions of the three gases and reported that 40:30:30 of CO₂:O₂:N₂ is the optimum gas mixture both to reduce Campylobacter and to extend shelf-life in poultry filets. The threshold CO₂ concentration that critically affects the viability of HAT C. jejuni has not been examined, and its determination still awaits future studies for the development of optimal gas mixtures of MAP to control HAT C. jejuni in poultry meat.

Our previous study revealed that most HAT C. jejuni strains belong to MLST CC 21 (Oh et al., 2015a), the major MLST CC...
implicated in human gastroenteritis ( Nielsen et al., 2010 ). It is possible that strains of C. jejuni with increased aerotolerance may survive well in foods and are more likely to reach humans, consequently causing human illnesses more frequently than aerosensitive C. jejuni strains. At this stage, it remains unknown why HAT C. jejuni strains harbor more virulence genes than oxygen-sensitive strains. In this study, we did not provide empirical evidences about the virulence, such as invasion of and adhesion to epithelial cells, and such works will be done in future studies. Nevertheless, this study also showed that MAP using CO₂ may be an interesting approach to control HAT C. jejuni in poultry meat.

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AUTHOR CONTRIBUTIONS

Design of the project: EO and BJ. Performance of the experiments: EO. Data analysis: EO, LM, LC, and BJ. Writing of the manuscript: EO, LM, LC, and BJ.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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