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Investigation of the Effects of Antimicrobials to Control *Campylobacter Jejuni* in Chicken Carcass

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Abstract

The aim of this study was to investigate the effects of antimicrobial substances to reduce *Campylobacter jejuni* (*C. jejuni*) ATCC 700819 in chicken carcass at the final wash or cooling step in the poultry slaughtering process. The inoculated chicken meat was divided into four groups, which were control, 5% nisin, 50% whey, and 5% *Nigella sativa (N. sativa)*. For both dipping and spray treatments, the microbial count of *C. jejuni* was analysed on 3 d, 4 d, and 7 d. The results showed that whey and *N. Savita* at 3 and 4 days, respectively, were more effective for spray method, whereas for nisin, it was more effective for dipping method at 3 and 7 days. For polymerase chain reaction (PCR), all isolates belonged to the genus *Campylobacter* and *C. jejuni*, as indicated by bands at 650 bp and 323 bp, respectively. For the enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR), it indicated that the isolated strain of *C. jejuni* was the same as to the reference strain of *C. jejuni* ATCC 700819 that was inoculated. The findings clearly suggested that the dipping and spray methods had the ability to reduce *C. jejuni* in chicken meat. To date, no effective, practical measures to reduce or completely prevent *C. jejuni*. This is the first report investigating the effects of antimicrobial substances (nisin, whey, *N. sativa*) on *C. jejuni*. Overall, this study should open the door for commercial companies to use alternative antimicrobial, which can be more competitive than those of commercial types.

Keywords: Chicken Meat, *Campylobacter jejuni*, Antimicrobials, PCR/ ERIC-PCR, Foodborne Illness.

INTRODUCTION

Many pathogenic species of bacteria are associated with chicken meat that also, degrade the quality of sensory and shelf-life. Chicken meat is classified as extremely sensitive meat because of its higher susceptibility to the microbial spoilage as it works as a perfect

medium for the growth of microbes (Raeisi et al., 2016). In recent years, the key factor in higher campylobacteriosis may be the higher popularity of chicken-derived white meat, in comparison with the red meat derived from both large and small ruminants (Lin, 2009).

Campylobacter species, especially *Campylobacter jejuni*, is one of the most common causes of foodborne bacterial gastroenteritis in humans worldwide (Bronzwaer et al., 2009). Chickens are a natural host for *C. jejuni*, and colonised commercial broiler chicken is the main vector for transmitting this pathogen to humans (Hermans, Van Deun, Martel, et al., 2011). It has been previously reported that both the meat products and the meat itself promote the growth and survival of *C. jejuni* (Balamurugan, Nattress, Baker, & Dilts, 2011). The handling of raw broiler meat or eating chicken meat that is not properly cooked have been shown to be associated with a higher prevalence of campylobacteriosis (Lindmark et al., 2009). Although from 50% to 80% of the broiler are potentially contaminated by *Campylobacter* spp.; the consumption, preparation, and handling of meat derived from broilers could cause up to 20% to 30% of campylobacteriosis (BIOHAZ, 2010). In several countries, the prevalence of *Campylobacter* spp. ranges from 90%–100% in retail chicken products (MADDEN, MORAN, SCATES, MCBRIDE, & KELLY, 2011). The main pathogens associated with illnesses are norovirus, *C. perfringens*, *Campylobacter spp.*, and nontyphoidal *Salmonella spp.*, representing 90% of the total pathogen-specific illnesses (Thomas et al., 2013). It was learnt that *C. jejuni* is a major risk factor to human health, where the acute symptoms of *C. jejuni* in humans are abdominal pain, fever, and diarrhoea (Abay et al., 2014). In this regard, the post-harvest treatment could be a promising control strategy in the production unit but it has yet to be explored (Zampara et al., 2017). Neither the modern hygienic practice in slaughterhouses nor the higher level of biosecurity has proven enough on their capability to control *C. jejuni* contamination in poultry meat (Zampara et al., 2017). Interestingly, the use of additives with antagonistic properties was found to be an excellent alternative to the direct use of antibiotics (Healy, 2014). The risk of contracting the infection by consumption and contact with the poultry meat is indicated by all of the results based on the hypothesis that the same genotype of poultry is being exposed to the humans (Ahmed et al., 2015). In the last few years, the ERIC-PCR technique has been used extensively in order to determine the epidemiology of *C. jejuni* at molecular level in different foods (Aydin et al., 2007). Throughout the evisceration process, there were many hurdles with the antimicrobial properties, which reduced the microbial load of broilers brought to the slaughter facility, in the reduction and prevention of contamination. This work utilised several types of antimicrobial substances (nisin, whey, and *Nigella sativa*). Nisin is classified as an antibiotic substances, where it is approved by World Health Organization (WHO) and Food and Drug Administration (FDA) as a Generally Recognized as Safe (GRAS) food additive (Guerra et al., 2005). Regarding whey, the lactoferrin is mainly present in milk and milk products at a level of

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up to 2% (Keri Marshall, 2004). The key physiological function of lactoferrin is to create a microbiostatic pressure on microorganisms that are sensitive to iron scarcity in the digestive tract through higher affinity for its binding with iron (Hill & Newburg, 2015). As for *N. sativa*, it was found that active compounds, such as melanin and thymohydroquinone, are the major constituents of the antimicrobial properties of *N. sativa* (Al Yahya, 1986). Overall, the aim of this study is to determine the effects of these antimicrobial substances on the survival of *C. jejuni* ATCC 700819 in chicken carcass after treatment.

MATERIALS AND METHODS

This study to verify the survival of *C. jejuni* after treatment with T1: control, T2: 5% nisin, T3: 50% whey, and T4:5% *N. sativa* after dipping and spraying treatments**.**

2.1 Experimental Design

The experiment was conducted at a laboratory in Erciyes University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Kayseri, Turkey. The experiment was performed in two stages (dipping and spraying).

The chicken meat was purchased from a commercial retail in Kayseri, Turkey. Firstly, the chicken meat was disinfected with alcohol 70%. Then, the chicken fillets were inoculated with reference strain, *C. jejuni* ATCC 700819, from which was cultured onto *Campylobacter* blood-free selective agar base blood agar (Oxoid, CM 271 B, UK) (mCCDA) (Merck KGaA/Germany), which already contains mCCDA (Microbiology CCDA Selective Supplement CCD/Merck/Germany) and supplement plate at 42°C for 24-48 hours under microaerobic condition The incubation of *C.jejuni*was applied with (Microbiology Anaerocult (Merck KGaA/Germany) inside a Microbiology Anaerobic Jar of 2.5 L (Merck KGaA/Germany), which was then incubated at 42°C for 48h under microaerobic conditions (5% O_2, 10% CO_2,85% N_2) (INCUCELL/Germany). Subsequently, a 0.5 McFarland of bacterial cell suspension was prepared and the samples were mixed gently before they were kept at room temperature for 5 min in a biosafety cabinet for bacterial attachment. The whole room had been previously sterilised by using ultraviolet light sterilisation (UV lamp). Then, each meat was divided into four groups. One of the meat was put in the control group, while the remaining three pieces of meat were treated with different treatments: nisin, whey, and *N. sativa* at different concentrations of 5%, 50%, and 5%, respectively, through spraying and dipping methods. Finally, the samples were washed with distilled water to stop the effect of added treatments.

All of the treatments comprised three replicates of chicken meat, where they were stored in the refrigerator at 4°C for 0, 3–4, and 7 days. The microbiological analyses of the

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samples of *C. jejuni* cells were carried out on days 0, 3–4, and 7 days. The sample homogenates were prepared on the day of analysis, where the meat was washed in the stomacher bag with buffered peptone water (Oxoid, CM0509, UK). Then, the sample homogenates were serially diluted -fold and 100 µl from each dilution was plated onto each plate that was inoculated to the modified charcoal-cefoperazone-deoxycholate-agar support containing antibiotic supplement. The bacteria were counted after a 48-h incubation at 42°C under microaerobic conditions. For our culture of the isolates the colonies were then transferred into blood agar (Oxoid, UK) and incubated for 48 h under microaerobic conditions at 42°C and the colonies were taken to crytube contains Brucella broth and 10% glycerol (Merk Germany) under freezing - 80°C.

2.2 Reference Strain

Campylobacter jejuni ATCC 700819 was used as a reference strain in all stages of the study.

2.3 Treatment of Stock Solution

The nisin powder was purchased from Sigma-Aldrich, where it was produced by *Lactococcus lactis* 2.5% (balance sodium chloride and denatured milk solids) (N5764-5G), with series number (1-Lot*057M4073V, 2-Lot*126M4013V, 3-Lot*126M4013V, 4-Lot*057M4073V) (Figure 2.1). An amount of 5000 mg of nisin powder was dissolved in 100 ml of HCl (0.02N) to obtain a concentration of 32500 IU/ml, which was then kept frozen at –20°C until use.Meanwhile, the fluid whey was obtained from the cheese plant in Erciyes University (Safiye Cıkrıkcıoglu Vocational School, Kayseri University, Kayseri, Turkey). The components of whey were fat (0.10), protein (2.44), total solids (6.48), water (27.5), density (1.0233), freezing point (0.403), and pH (6.25). The *N. sativa* essential oil was purchased from the famous herb market in downtown.

2.4 Preparation of *Campylobacter jejuni* ATCC 700819 Suspension

One ml of the bacterial suspension of *Campylobacter jejuni* ATCC 700819 was diluted with sterile tryptic soy broth (TSB). Then, it was serially diluted in 10-fold, plated on the mCCD agar plates, and incubated for 48 h under microaerobic conditions at 42°C. After incubation, the grown colonies were counted to calculate the CFU(Smith et al., 2008).

2.5 | Molecular Techniques

In this study, the samples were isolated, cultured, and identified, in which the colony morphology was confirmed under the microscope.

Taking colonies that were grown on the blood agar based on their confirmed observation, only ten colonies from different treatments were examined after DNA extraction and identification using the PCR and ERIC-PCR techniques.

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2.5.1 Culture

To obtain DNA isolation and PCR, the colonies were taken to identify its *C. jejuni*, which was transferred into blood agar and incubated for another 48 h under microaerobic conditions at 42°C.

2.5.2 DNA Isolation

For the molecular identification of *C. jejuni* isolates, DNA extraction from identified isolates was performed according to the manufacturer's guidelines using a Bacterial DNA Extraction Kit and InstaGene^{TM}Matrix (Bio-Rad Laboratories, Hercules, USA).

The procedure began with choosing a bacterial colony and resuspend it in 1 ml of distilled water in a microfuge tube. Then, it was put in a centrifuge for 1 min at 12000 rpm, removing the supernatant. Subsequently, 200 µl of InstaGene^{TM} matrix was added to the pellet at 56°C for 30 min and it was vortexed at high speed for 10 s. The tube was put in a 100°C heat block for 8 min and it was also vortexed at high speed for 10 s and Spin at 12000 rpm for 3 min. Then, 20 µl of the resulting supernatant was used per 50 µl of PCR reaction, where the remainder of the supernatant was stored at –20°C.

2.5.3 Polymerase chain reaction (PCR)

A modified version of an PCR assay(Wang et al., 2002)was used to identify members of the genera *Campylobacter* spp. and *C. jejuni*. Each PCR tube contained 200 µm deoxynucleotide triphosphate; 2.5 µl of 10× reaction buffer (500 mMTris-HClat pH 8.3, 100 mM KCl, and 50 mM $(NH_4)_2SO_4$); 20 mM $MgCl_2$; 0.5 µm *C. jejuni*, and 0.2 µm 23S rRNA primer. 1.25 U of Fast Start Taq DNA Polymerase (Roche Diagnostics, GmbH, Mannheim, Germany) and 2.5 µl of whole-cell template DNA. The volume was adjusted with sterile distilled water until a volume of 25 µl was attained. DNA amplification was carried out in Thermal Cycler (Thermo using an initial denaturation step at 95°C for 6 min, followed by 30 cycles of amplification (denaturation at 95°C for 0.5 min, annealing at 59°C for 0.5 min, and extension at 72°C for 0.5 min), ending with a final extension at 72°C for 7 min. The primer sets were evaluated individually for primer specificity using the reference strain, *C. jejuni* (Wang et al., 2002).

2.5.4 Enterobacterial Repetitive Intergenic Consensus (ERIC-PCR)

ERIC-PCR and electrophoresis were carried out using the method (Houf, De Zutter, Van Hoof, & Vandamme, 2002). Briefly, the total of 50 µl volume of prepared PCR mixture consisted of 5 µl of 10× PCR buffer, 8 mM $MgCl_2$, 5 U/µlTaq Polymerase, dNTP Mix at a final concentration of 0.2 mM , 25 pmol/µl with primers 1R (5'-ATGTAAGCTCCTGGGGATTCAC-3'), 2R (5'-AAGTAAGTGACTGGGGTGAGCG-3') (Houf et al., 2002), and 1 µl DNA target solution. The DNA amplification consisted

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of an initial denaturation at 94°C for 5 min, followed by 40 cycles at 94°C for 1 min, 25°C for 1 min, and 72°C for 2 min.

2.6 Statistical Analysis

All experiments were performed using three biological replicates. The statistical differences between the means of the treatment group (quantitative variables) were analysed by one-way ANOVA, followed by multiple pairwise comparisons (Tukey's test, α=0.05). For statistical analyses and graphs, SPSS version 20.0 and GraphPad Prism were used. Data of the three biological replicates were expressed as mean ± SD, where the differences were considered significant at p<0.05.

3 RESULTS

3.1 Survival of *Campylobacter jejuni* ATCC 700819 After Treatment with Dipping and Spraying *invitro*

3.1.1 Survival of *Campylobacter jejuni* after Dipping Treatment

The results after a four-day isolation from the chicken meat stored in the refrigerator at a 4°C have illustrated that the T2 treatment (3.51 log CFU/g) displayed the highest statistical significance at p< 0.01 in all treatments. Meanwhile,T1 (4.60 log CFU/g) and T4 (4.56 log CFU/g) treatments recorded the lowest statistical significance at p< 0.01 (Graph 1.1).Regarding the results for seven days, where the control treatment T1 (0.00 log CFU/g) recorded the highest statistical significance at p< 0.01, the colonies of *C. jejuni* were shown to have declined to an undetectable level for fresh chicken meat, following seven days of storage at 4°C. On the other hand, T3 recorded the lowest statistical significance at p< 0.01 (4.36 log CFU/g) (Graph 1.2). As illustrated in Graphic 3.3, comparison between time and treatment as immersion on the survival of *C. jejuni* was done from 96 h to 168 h. The interaction between the concentration of the antimicrobials and treatment time contributed to growth inhibition of *C. jejuni* over 96 h to168 h, illustrating significant differences, except for T3 (50% whey).

3.1.2. Survival of *Campylobacter jejuni* after Spraying Treatment

The results for three days illustrated that T3 (50% whey) was significant at p< 0.05, as the number of bacteria was reduced by one-logarithmic reduction cycle (4.04 log CFU/g), compared to that of the control treatment (5.03 log CFU/g)(Graph 1.4).Regarding the results for four days, it was illustrated that T4 (3.65 log CFU/g) recorded the highest statistical significance at p< 0.01, while T1(4.47 log CFU/g) and T3 (4.36 log CFU/g) recorded the lowest statistical significance at p< 0.01 (Graph 1.5). However, for the seven-day result, it was found that the colonies of *C. jejuni* were shown to have declined to an undetectable level for fresh chicken meat, following seven days of storage at

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4°Cthat occurred three times. The interaction between the concentration of the antimicrobials and treatment time contributed to variable growth inhibition of *C. jejuni* for all treatments numerically but it is not significant for T4 (*N. sativa*), as illustrated from the statistical significant differences at $p< 0.05$ over 72 and 96 h to 168 h (Graph 1.6).

3.2 Molecular Identification and Control of *Campylobacter jejuni* ATCC 700819

In this study, samples were cultured and identified through colony morphology, as confirmed through microscopic observation. Colonies that were grown on blood agar were taken, depending on their shape. All colonies were examined and identified via PCR in detecting isolated colonies by using the ERIC-PCR technique for control and investigation.

All isolates were positive at 650 bp and 323 bp, where 650 bp gave identify to the *Helicobacter* spp., *Arcobacter* spp., and *Campylobacter* spp.;while323 bp gave identify to *C. jejuni* (Figure 3.1). In addition, all isolates of *C. jejuni* had the same ERIC-PCR profiles. From the analysis, it showed that isolates were the same (Figure 3.2).

4 DISCUSSION

A hard-to-treat chronic bacterial infection is one of the most significant challenges to conventional antibiotic therapy. Although majority of the chicken meat is cooked through heating during the preparation phase, which actively inactivates the *Campylobacter* spp., however, half of the chicken livers are consumed raw, which may contain active *Campylobacter* spp.(Evers, van der Fels-Klerx, Nauta, Schijven, & Havelaar, 2004). Although the optimum range of temperature is 37–42°C for the growth and development of *Campylobacter* spp., but the physiological activity of *C. jejuni* has been observed even at 4°C (Hazeleger, Wouters, Rombouts, & Abee, 1998). Therefore, only freezing or refrigeration is not recommended to prevent the prevalence of *C. jejuni* outbreak. These temperatures were chosen as a fresh chicken is kept refrigerated at 4°C in supermarkets and domestic setting. It has been suggested that it is more logical to take control measures to reduce the level of *Campylobacter* spp. in broiler meat during the time of processing, rather than developing a broiler flock that is resistant to this organism (Hermans, Van Deun, Messens, et al., 2011). The selection of temperature was a vital parameter in this study. It has been reported that the *C. jejuni* can survive in a wide range of temperature, starting from 4°C to –20°C(El-Shibiny, Connerton, & Connerton, 2009). It has been shown that the survival rate of *C. jejuni* is higher in chicken juice in comparison without it at 5°C(Birk, Ingmer, Andersen, Jørgensen, & Brøndsted, 2004). Immediately after slaughter and processing, the chickens were typically stored at 4°C at the processing centre and then transported to the supermarket. In supermarkets, chicken products typically stay for 4–7 d before selling. Furthermore, the chickens may wait in the

consumers' refrigerator for another few days. Therefore, three time points (3, 4, and 7 days) were chosen to examine after inoculation of the *C. jejuni* in raw chicken meat.

The results obtained from this study observed a lower survival ability of *C. jejuni* in nisin treatment after 4 d of the dipping treatment, compared to other treatments. This finding is inagreement with another study, in which after 15 min of dipping treatment, there was a significant log reduction in the nisin group, compared to the control and other groups (Long & Phillips, 2003).

In addition, the result of the spraying experiment with nisin at 3 d revealed that there was no significant reduction in the survival of *C. jejuni*, compared with other treatments. The spraying method is illustrated to be more efficient in removing the surface microorganism, as compared to that by dipping method. However, at day 4-time point, it was observed that there was no significant reduction of the survival rate of *C. jejuni* compared to other treatments. Surprisingly, the nisin spray treatment was unable to reduce the survival of *C. jejuni*, compared to control group at day 4 of the treatments. In addition, nisin showed a dramatic increase in activity from 4 d to 7 d, causing in the reduction of survival of *C. jejuni* in the chicken meat. On the other hand, Khalafalla *et al*.(2016) found that there was a slight increase in the survival rate of mesophilic count after 7 d compared to after 4d (Khalafalla, Hassan, Ali, & Hassan, 2016). Moreover, this study showed that whey had significant effects on the reduction of survival of *C. jejuni*, compared with *N. sativa* and control groups after 4 d of dipping treatment. Nevertheless, at 7 d of dipping treatment, there was no significant reduction in the survival of *C. jejuni*, compared to all treatments. This study showed that some components of whey, such as the irons cavengers lactoferrin, have antimicrobial activity (Orsi, 2004). In fact, there was shortage of information about timepoint investigation based on whey treatment to reduce the *C. jejuni* activity. Hence, a correlation between the 1results of this study with others was not established. On the other hand, the spray treatment at 3 d presented significant reduction in the whey group, compared to the control. This finding is inagreement with Naidu (2002),where it was reported that whey contains an active compound called lactoferrin that has the ability to reduce bacterial content on the surface of the meat. Whey did not show any significant reduction of *C. jejuni*, compared to other treatments on day 4 of spray treatment.

The results of this study indicate that *N. sativa* did not show any significant differences, compared to other treatment groups at 3 d. Moreover, lower survival ability of *C. jejuni* was also observed in *N. sativa* treatment after 4 d of spray treatment, compared to control and whey treatments. Furthermore, a significant reduction was observed in the survival of *C. jejuni* in chicken meat treated with *N. sativa* at day 4-time point compared to day 3-time point. From 3-day to 4-day time point, *N. sativa* showed the ability to decrease the survival rate from log 3.65 CFU/g, compared to that of control group (log 4.47 CFU/g). It

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is quite interesting to note that at day 3-time point, no significant reduction of *C. jejuni* count was observed in the chicken meat treated with *N. sativa*, compared to control group. It is well known that *N. sativa* contains many bioactive molecules, which are effective in reducing the microbial content in poultry system. Unfortunately, most studies investigated *N. sativa* with the dietary intake of oil extract and their impact on growth and production performance. The results obtained from the dipping treatment at 7 d showed there was not any indication of bacterial survival in the control group, which means the bacterial count was null in the control group. It seemed that the level of *C. jejuni* had been decreased to a very low or undetectable level on fresh chicken meat when the meat was stored at 4°C for 7 d. In fact, this experiment was repeated three times and similar results were obtained each time. It has been shown that the infection caused by *C. jejuni* did not require antibiotic treatment characteristics in most human gastroenteritis cases (Acheson & Allos, 2001). The reason may be due to VBNC, where VBNC cells (the state describes a subpopulation of bacteria that have transiently lost their ability to grow on routine laboratory media on which they were previously able to grow) may have lost its culture ability over time but a portion of the original population remains viable during a seemingly "lethal" stress (Ayrapetyan, M., Williams, T., & Oliver, 2018). The use of PCR is a common practice for identifying *Campylobacter* spp. and *C. jejuni*. In this experiment, the PCR system was employed to detect the *Campylobacter* spp. The results of the PCR revealed that all isolates were positive, in which this was confirmed by the presence of two distinct bands after PCR. The approximate length of one band was650 bp and that of the other was around 323 bp. The 650 bp and 323 bp band indicated *Campylobacter* spp. and *C. jejuni*, respectively (Figure 3.1). Similar method was used to identify *Campylobacter* spp. and *C. jejuni* and other isolates obtained from the chicken slaughterhouse samples (Wang et al., 2002).

In addition, definitive results are sometimes unattainable at species level. Therefore, it is advisable to add another identification technique for faster identification through a genotypic technique. In order to uncover the biological characteristics of *C. jejuni* isolate, genotyping was performed using ERIC-PCR typing and PCR, as they are a simple and rapid typing method for the identification of marked heterogeneity or otherwise. Genotyping using ERIC-PCR produces strain-specific fingerprinting, which can further be used in the evaluation of the *C. jejuni* genetic diversity (Hulton, Higgins, & Sharp, 1991). ERIC-PCR is a good approach for molecular typing of the *Campylobacter* species. In the current study, selected colonies were analysed using ERIC-PCR method to type the different species in the colony. The results showed that there were no significant differences in the ERIC-PCR method in the samples analysed in both studies, as all isolates were positive. The analysis performed showed that all isolates were similar, therefore, there was no genetic variation (Genetic Diversity).

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4 |CONCLUSIONS

A big concern regarding processing poultry that is contaminated with *C. jejuni* at the processing plants is preventing cross-contamination of the poultry meat. The risk factors include slaughter, dressing, and processing. Both pre- and post-slaughter intervention strategies play an important role. The results of this work showed that when dipping method was used, nisin was the best selection. Meanwhile, for spraying method, the whey and *N. sativa* had better controlling ability in reducing the survival of *C. jejuni* in the chicken meat. These results showed that it is very likely that the chicken meat could be naturally contaminated with *C. jejuni*, in which this situation may persist even when the meat is stored in the refrigerator. *C. jejuni* has developed resistance to several antimicrobials and possesses the mechanism to survive under hostile environments. To date, there are still no effective, safe, and practical control measures to reduce or completely prevent *C. jejuni* colonisation.

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Competing Interests

There is no present conflict of interest by the author of neither this research nor a declared conflict in researching this article and their findings.

Graphical Table of Contents

Article Title	**Investigation of The Effects of Antimicrobials to Control *Campylobacter jejuni* in Chicken Carcass**
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Text Summarizing	- Our results showed that, if the dipping method is used nisin is the best choice. While regarding spray, the whey and *N. sativa* a better controlling ability to reduce the *C. jejuni* in the chicken meat. - These results showed that it is very likely that chicken meat could naturally contaminated with *C. jejuni* and may retain even if stored in the refrigerator. - *C. jejuni* has developed resistance to several antimicrobials and possesses the mechanism to survive under hostile environments.
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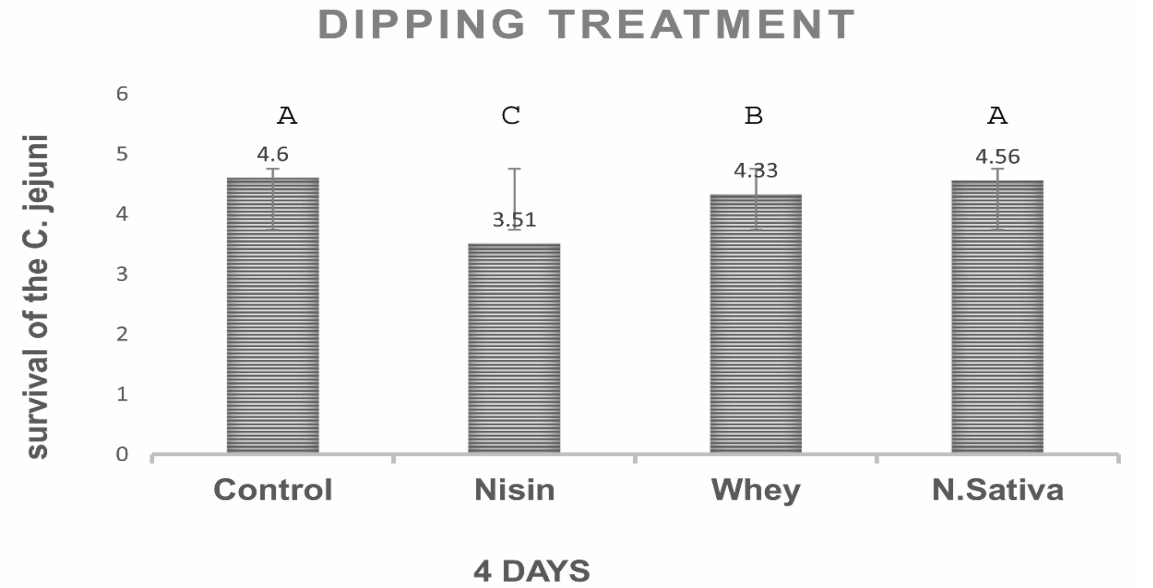

∗ Experimental treatments as dipping: [T1 = control, T2 = Nisin 5%, T3 = Whey 50%, T4 =N. *Sativa* 5%].

∗ Symbols of character indicate significant.

Graphic 1.1. The results of isolated *C. jejuni* ATCC 700819 from chicken meat were treatment as dipping and stored for 4 days at refrigerator at 4° C *in vitro.*

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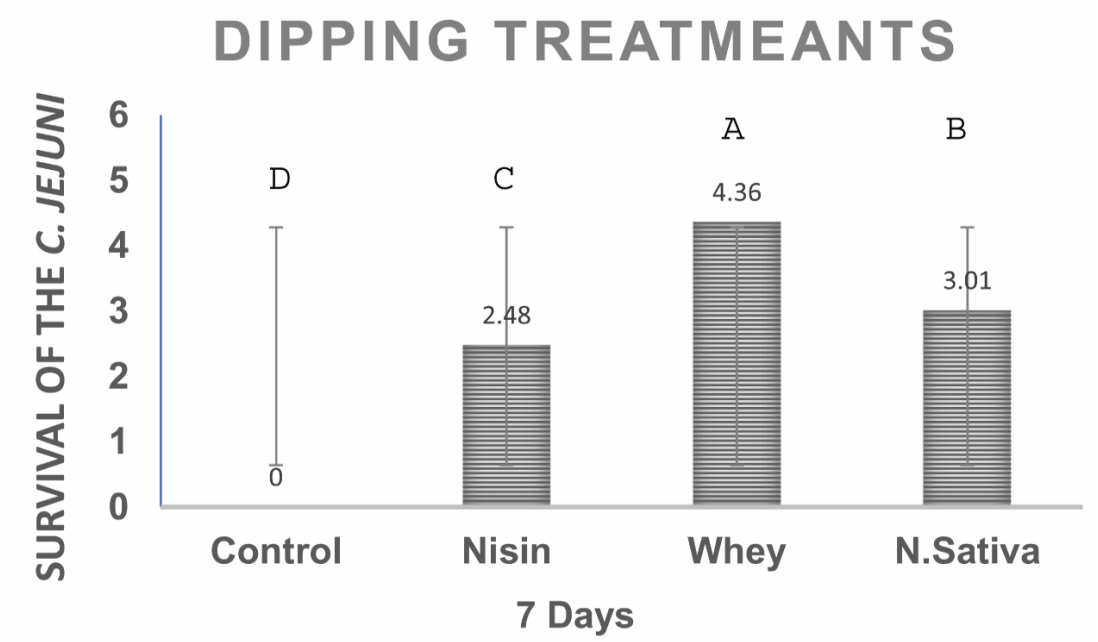

* Experimental treatments as dipping: [T1 = Control, T2 = Nisin 5%, T3 = Whey 50%, T4 =N. *Sativa* 5%].

* Symbols of character indicate significant.

Graphic 1.2. The results of isolated *C. jejuni* ATCC 700819 from chicken meat were treatment as dipping and stored for 7 days at refrigerator at 4° C *in vitro.*

* Experimental treatments as dipping: [T1 = Control, T2 = Nisin 5%, T3 = Whey 50%, T4 =N. *Sativa* 5%].

* The significant {(A, B), (A, B), (A, A), (A, B)} for (4-7) d. for {(Control, Nisin, Whey, N. *Sativa*). as respectively}.

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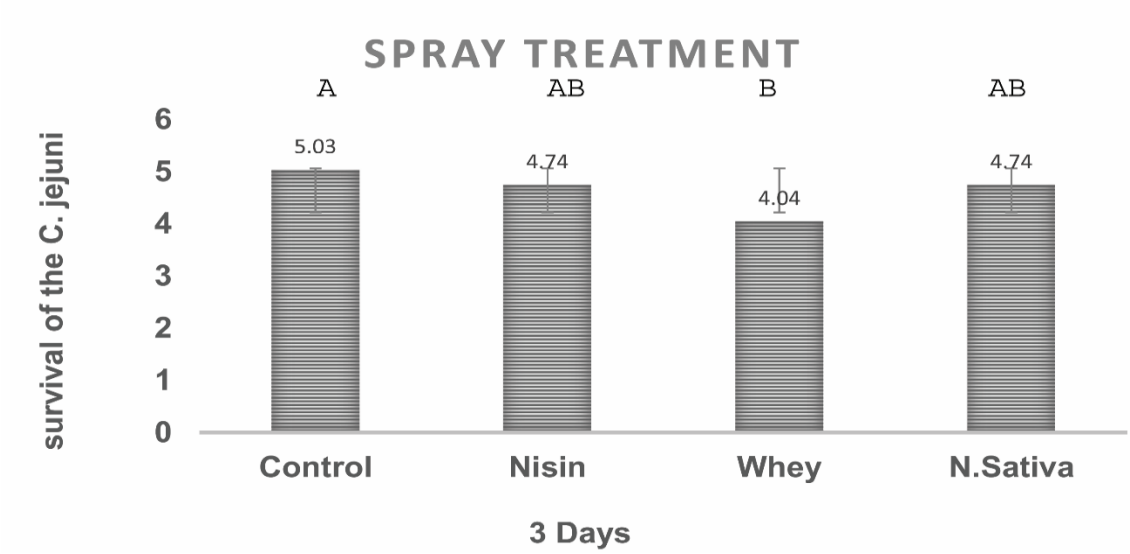
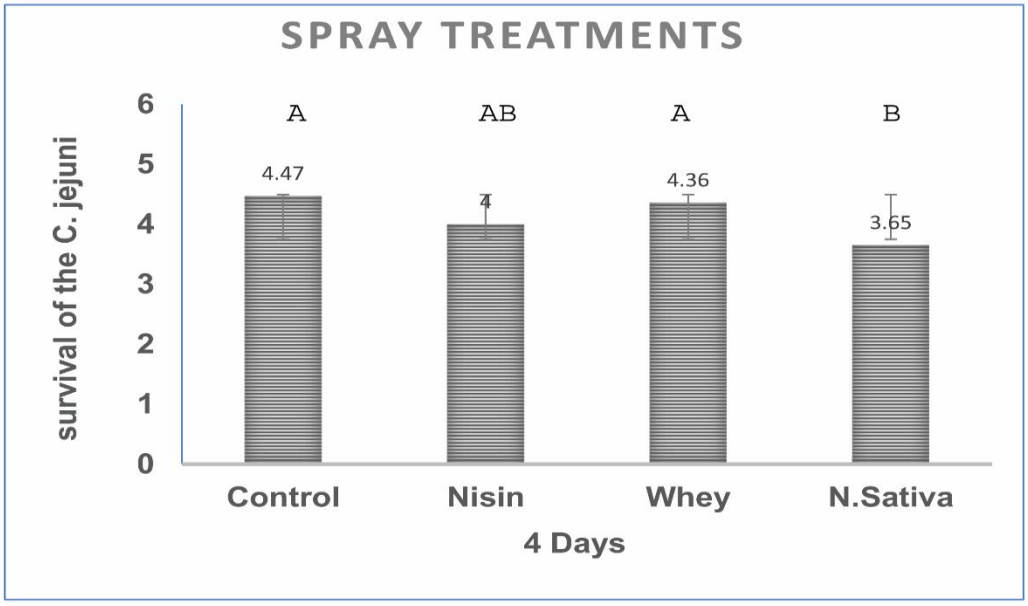
Graphic 1.3. Time-kill curve of treatments against *C. jejuni*



- Experimental treatments as spray: [T1 = control, T2 = Nisin 5%, T3 = Whey 50%, T4 =N. *Sativa* 5%].
- Symbols of character indicate significant.

Graphic 1.4. The results of isolated *C. jejuni* ATCC 700819 from chicken meat were treatment as spray and stored for 3days at refrigerator at 4°*C in vitro.*



- **Experimental treatments as spray: [T1 = control, T2 = Nisin 5%, T3 = Whey 50%, T4 =N. *Sativa* 5%].**
- **Symbols of character indicate significant.**

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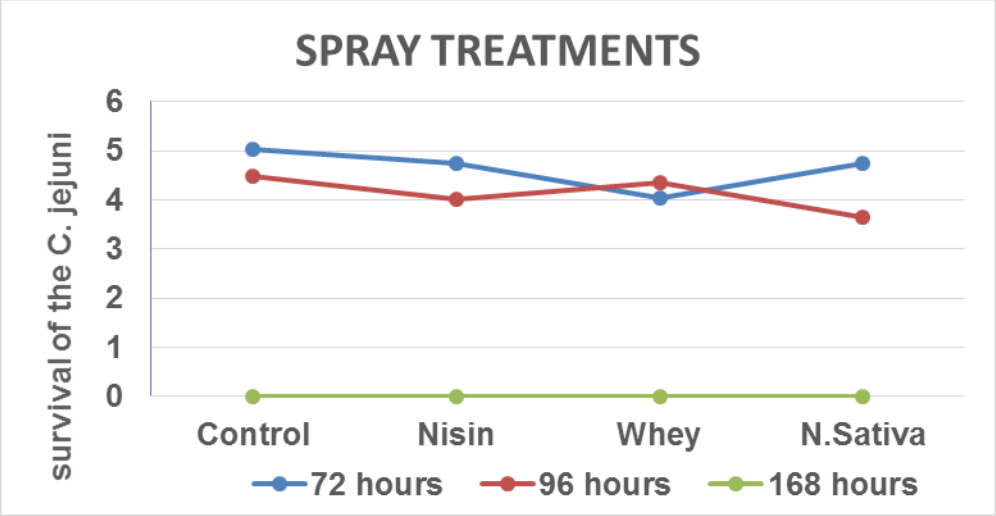
Graphic 1.1. The results of isolated *C. jejuni* ATCC 700819 from chicken meat were treatment as spray and stored for 4 days at refrigerator at 4°C *in vitro.*



* Experimental treatments as dipping: [T1 = control, T2 = Nisin 5%, T3 = Whey 50%, T4 =N. *Sativa* 5%].

* The significant {(A, A), (A, A), (A, A), (A, B)} for (3-4) d. for {(control, Nisin, Whey, N. Sativa). as respectively}.

Graphic1.2. Time-kill curve of treatments against *C. jejuni*

Figure 2.1. The can of nişin-Sigma-Aldrich.

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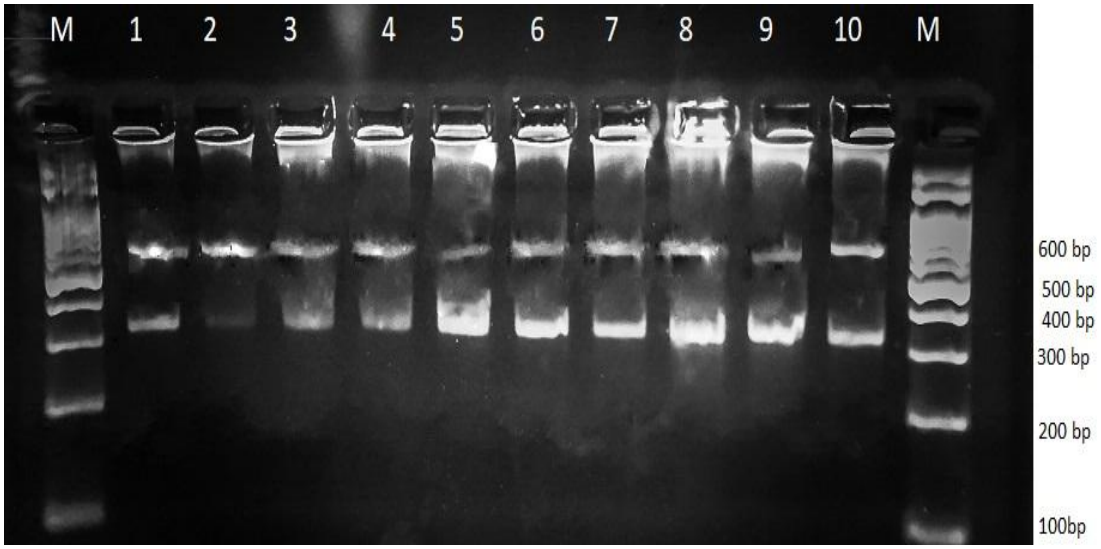

Figure 3.2. Identification of *Campylobacter* species isolates at the species level by PCR with1.5% agarose gel. Lanes: M, size marker (GeneRuler 100 bp DNA ladder; Fermentas); 1–10 samples.

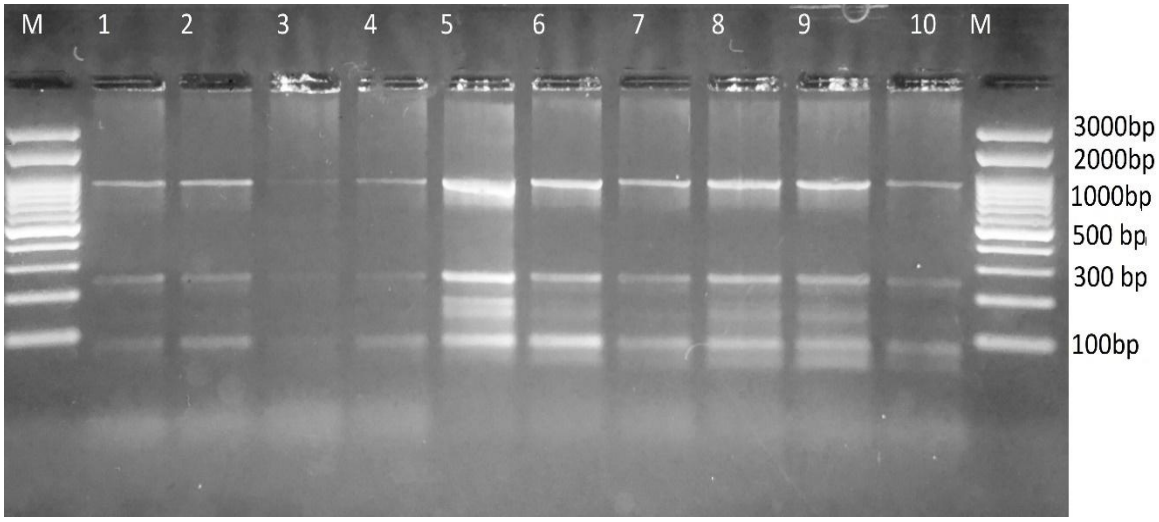

Figure 3.3. ERIC-PCR fingerprinting patterns of the *Campylobacter jejuni* isolates. Lanes: M, marker (GeneRuler 100 bp DNA ladder); 1–10 samples, electrophoresed on 2.0% agarose gel. Lane M: 100 bp DNA ladder (molecular weight in bp).