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Prevalence of *Campylobacter jejuni* in chicken produced by major poultry companies in Saudi Arabia

Hany M Yehia[1,2*] and Mosffer M AL-Dagal[1*]

Abstract

Background: Campylobacter is a foodborne pathogen that is commonly associated with chicken. The aim of this work was to evaluate the prevalence of *Campylobacter jejuni* (as affected by refrigerated storage) in chicken samples obtained from the wholesale poultry market in the northern part of Riyadh City, Saudi Arabia.

Findings: A gradual increase in the number of positive samples was noted during storage at 4°C. On days 1, 3, and 7, the number of positive samples were 10 (30.305%), 15 (45.45%), and 27 (81.81%), respectively. Of 99 tested samples, 52 (52.25%) were positive for *Campylobacter jejuni*. Protein profiling by Sodium dodecyl sulfate -Polyacrylamide gel electrophoresis (SDS-PAGE) was used to identify *Campylobacter jejuni*. The results were verified using Analytical Profile Index (API Campy system, Marcy l'Etoile, France). Forty-three (82.69%) positive isolates were identified as *C. jejuni subsp. jejuni* 2, 5 isolates as *C. jejuni subsp. jejuni* 1 (9.61%), and 4 isolates as *C. jejuni subsp*. doylei (7.69).

Conclusion: *C. jejuni* positive samples increased rapidly during storage at 4°C for approximately 1 wk. Our results also indicated a connection between the protein profiles on SDS-PAGE and API Campy used for the identification of *C. jejuni*.

Keywords: *Campylobacter jejuni*; Chicken; Refrigeration storage

Introduction

Campylobacter jejuni is a well-known food-borne pathogen, transmitted to humans by the eating of warm-blooded animal meat, especially poultry (Jay et al. 2005; Nielsen et al. 1997). This bacterium is a major cause of food-borne diarrhea in many countries (Crushell et al. 2004; Iovine et al. 2008. It has gained more attention in the last 30 years because it has been recognized as a major cause of human illnesses, ranging from gastroenteritis to Guillain-Barré Syndrome (Khanna et al. 1996; Tauxe, 2001; Moore et al. 2005). The 2 most frequently occurring *Campylobacter* species that are of clinical significance because of meat consumption and meat products are *C. jejuni* and *C. coli*. *Campylobacter jejuni* accounts for more than 90% of incidences of human campylobacteriosis (Lindmark et al. 2009). Campylobacteriosis in humans results from eating undercooked meat and/or contaminated meals (Corry and Atabay 2001). Survey studies have revealed a high prevalence of *Campylobacter* in poultry meats (Dickins et al. 2002; Ridsdale et al. 1998; Stoyanchev et al. 2007).

Few studies on *Campylobacter* in the Saudi Arabian food market have been performed. Therefore, this study investigates the prevalence of *Campylobacter jejuni* in locally produced refrigerated chicken carcasses, as affected by storage time. Whole-cell protein profiles of presumptive Campylobacter isolates were compared with the standard strain of *Campylobacter jejuni* ATCC 33291 on SDS page. High degree of similarity within standard strain was confirmed by biochemical identification using an API CAMPY biotyping identification system.

Materials and methods

Sample collection

Whole chicken carcasses (n = 99) were obtained from a wholesale poultry market located in the northern part of Riyadh City, Saudi Arabia. The samples were collected from 11 major national poultry companies (designated by the letters A through K). Nine samples were collected from each company. The samples were transported at

* Correspondence: hanyyehia43@yahoo.de; maldagal@ksu.edu.sa
[1]Food Science and Nutrition Department, College of Food and Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia
Full list of author information is available at the end of the article

refrigeration temperature to the Food Microbiology Laboratory, College of Food and Agricultural Sciences, King Saud University.

Experimental design

Refrigerated chicken samples were divided into 3 groups. The first group (including 33 samples, 3 samples **(3 runs)** per company) was tested for the presence of *Campylobacter jejuni* on Day 1 (the purchase date). Similarly, the next 2 groups (33 samples each) were tested for the micro-organism at Day 3 and Day 7 after the purchase date and stored in refrigerator at 4°C.

Isolation and identification of *Campylobacter*

Campylobacter jejuni was isolated according to the methods described by the Food Safety and Inspection Service (FSIS) (1998). Each carcass was rinsed in a sterile plastic bag with the addition of 200 mL of 0.1% peptone by manual shaking for 60 s. Ten milliliters of the rinse was centrifuged at 5000 rpm for 5 min, and 2 loops of the pellet were streaked on modified charcoal cefoperazone deoxycholate agar (mCCDA, Oxoid CM739, Basingstoke, Hampshire, UK) and *Campylobacter*-selective agar (Preston, Oxoid CM0689). Preston Campylobacter selective supplement (Oxoid, SR0117) and lysed horse blood (Oxoid, SR0048) were used for the selective isolation of *Campylobacter jejuni* from the samples (Figueroa et al. 2009). As a confirmation step, 10 mL of each rinse fluid was transferred to 90 mL of Preston enrichment broth (to prepare Preston Campylobacter selective enrichment broth, 12.5 g of Nutrient Broth No.2 (Oxoid CM0067) was dissolved in 475 ml of distilled water and sterilized by autoclaving at 121°C for 15 minutes. Twenty five 25 ml of Lysed Horse Blood (Oxoid SR0048), 1 vial of Preston Campylobacter Selective Supplement (Oxoid SR0117) and 1 vial of Campylobacter Growth Supplement (Oxoid, SR0232) were added to the cooled 475 ml medium. Five ml volumes were aseptically dispensed in sterile small screw-capped bottles and incubated at 37°C for 48 h in a gas mixture of BBL GasPak, 70304 (Becton Dickinson and Cockeysville, MD, USA), (5% O_2, 10% CO_2, and 85% N_2). The enrichment was streaked onto selective media, and the plates were incubated at 42°C for up to 48 h under microaerophilic conditions. After incubation, the plates were inspected for presumptive colonies before Gram staining, and cells resembling with *Campylobacter* were subcultured onto mCCDA by streaking colony method and incubated for 2 to 5 d at 42°C under microaerophilic conditions.

Polyacrylamide Gel Electrophoresis (PAGE)

The preparation of isolates for SDS-PAGE and the running of the samples were performed according to the method by Scarcelli et al. 2001. Electrophoresis was performed in a 12% polyacrylamide running gel and a 4% stacking gel, with a 0.025 M Tris 0.19 M glycine buffer pH 8.3, and 100 μL of a sucrose buffer (50 mM Tris–HCl, pH 8; 40 mM EDTA, pH 8; 0.75 M sucrose).

Preparation of cell extract

An overnight culture (100 μL) was inoculated into a 10 ml of fresh medium (Brain heart infusion-Oxoid, CM1135) and grown to an Optical Density (OD) 620 of 0.6 to 0.8 (3 to 4 h). The cells were collected and weighed, and 250 mg of cells were then suspended in 100 μL of a TES buffer (50 mM tris HCl, pH 8, 1 mM EDTA, 25% sucrose). Twenty microliters of lysozyme (50 mg/mL) and 5 μL mutanolysin (5000 u/mL) were added to the suspended cells in the TES buffer and incubated at 37°C for 30 min. Five to ten microliters of 20% SDS were added, and the contents were mixed until they became clear visible. The contents were stored at −20°C for 1 to 2 d (Ismail 2007).

Fifty-microliter extracts (standard and isolated bacteria) were loaded on SDS-PAGE. Electrophoresis was performed at 25°C in a vertical tank apparatus using a constant-voltage power supply, until a bromophenol blue tracking dye reached the bottom of the gel. Gels were stained with 0.25% Coomassie Brilliant Blue R-250 (Bio-Rad, Marnes-la-Coquette, France) in water: methanol: acetic acid (6.5:2.5:1) for 18 h at room temperature. Gel destaining was performed by continuous agitation in a methanol: acetic acid: water (20:10:70 v/v/v) solvent until obvious bands of proteins were obtained.

Whole-cell protein profiles of presumptive *Campylobacter* isolates were compared with the standard strain of *Campylobacter jejuni* ATCC 33291 on SDS-PAGE. A high degree of similarity with the standard strains was confirmed by biochemical identification using an API CAMPY biotyping system and a catalase test.

Biochemical identification of *Campylobacter* isolates

BioMérieux API CAMPY was used according to the manufacturer's instructions for the biochemical identification of *Campylobacter*. The BioMérieux API CAMPY strip consisted of 20 microtubes containing dehydrated substances, with each microtubule corresponding to an individual test. The 20 tests were divided into 2 parts; the first part was composed of enzymatic and conventional tests, and the second part comprised assimilation or inhibition tests (Gorman and Adley 2005). The results of the enzymatic tests were obtained with the addition of conventional reagents after 24 h of incubation at 37°C under aerobic conditions. The results of the assimilation and inhibition tests were recorded after 24 h at 37°C under microaerophilic conditions. Incubation was extended to 48 h if the succinate assimilation test was negative, as indicated by the manufacturer's instructions.

Results and discussion

Table 1 shows the prevalence of *C. jejuni* in refrigerated chicken samples, as affected by storage time. According to the table, the samples from all 11 poultry companies contained *C. jejuni* at one or more of the tested shelf-life dates. At Day 1 of the shelf life, *C. jejuni* was recovered from 30.30% of the tested samples (10 of 33 samples). This percentage increased to 44.45% and to 81.81%, after refrigeration for 3 d and 7 d, respectively, indicating the positive effect of natural enrichment. Poultry companies B, C, I, and J had included highly positive samples for *C. jejuni* (n = 6; 66.7%), followed by A and E (n = 5; 55.6%), and then D, F, and K (n = 4; 44.4%). Only one sample (n = 1; 11.1%), from company G, was positive.

Survival of *C. jejuni* at refrigeration temperature approved in one study. The outcome of this work agreed with other research works indicating that the survival of *C. jejuni* at refrigeration temperature range (4 to 7°C) is better than range (20 to 30°C), and that it exhibits greater survivability at chilled temperatures (Karenlampi and Hanninen 2004; El-Shibiny et al. 2009). Rollins and Colwell (1986) showed that, at 4°C, *C. jejuni* can survive and remain at a viable but non-culturable stage for approximately 4 mo. Zhao et al. (2000) showed that *C. jejuni* survive for days or weeks in refrigerated foodstuffs. Vashin and Stoyanchev (2011) established that the microorganisms did not grow in chilled or frozen meat, but are able to survive during the storage period at 1-4°C. Campylobacter were detected up to the 25^{th} days while at -18 to −20°C: up to the 45^{th} day. The juice released into the bags from poultry liquidation is highly nutritive and forms microaerophilic conditions suitable for *Campylobacter*. Birk et al. (2004) confirmed that compounds present in chicken juice protect, and thus, prolong the survival of *C. jejuni* during storage at refrigerator temperatures. They confirmed that incubation at 5°C extended viability of cells of *C. jejuni*, and incubation at 10°C significantly prolonged the viability of *Campylobacter*. In addition, they found that storage in chicken juice at both 5°C and 10°C significantly prolonged the viable cells of *C. jejuni* compared to incubation in reference media. The total number of samples tested positive for *C. jejuni* was 52 (52.52%). Similar results in a previous survey study (Rahimi and Tajbakhsh 2008) found 56.1% of chicken samples to be positive for *Campylobacter*. Few studies related to the occurrence of Campylobacter in Saudi Arabian food market, from 2002 to 2004, two studies were conducted to assess specimens obtained from slaughter-houses in Bahrain and Saudi Arabia for *Campylobacter* contamination. In one study, specimens consisting of 35 whole chickens, 27 chicken livers, and 38 chicken faeces were assessed using a combination of three culture methods, and just over half (57%) were found to be positive for *Campylobacter* contamination (Ghazwan 2006). In another study, 60 chicken faeces specimens were assessed using a newly developed multiplex PCR technique with 100% *Campylobacter* detection (Al Amri et al. 2007). However, in both studies, *C. jejuni* accounted for the majority of *Campylobacter* detected. The findings of this study, which is the first of its kind in our setting, indicates a need for

Table 1 Prevalence of *Campylobacter jejuni* in refrigerated whole chicken carcasses of 11 poultry companies in Riyadh City wholesale market

Poultry samples	Group 1			Group 2			Group 3			Total	%
	First day of the production date			3^{rd}days of the production date			7^{th} day of the production date			Positive	
	Run1	Run2	Run3	Run1	Run2	Run3	Run1	Run2	Run3	(out of 9)	
A	+	-	-	+	-	-	+	+	+	5	**55.6**
B	-	-	+	+	-	+	+	+	+	6	**66.7**
C	-	+	-	+	+	+	+	+	-	6	**66.7**
D	-	+	-	-	-	-	+	+	+	4	**44.4**
E	+	-	-	+	-	+	-	+	+	5	**55.6**
F	-	-	+	+	-	+	-	+	-	4	**44.4**
G	-	-	-	-	-	-	-	+	-	1	**11.1**
H	+	-	-	+	-	-	+	+	+	5	**55.6**
I	-	+	-	+	-	+	+	+	+	6	**66.7**
J	-	-	+	+		+	+	+	+	6	**66.7**
K	-	+	-	-	-	-	+	+	+	4	**44.4**
Number of total samples	**33**			**33**			**33**			**99**	
Number of positive sample	**10**			**15**			**27**			**52**	-
%	**30.30**			**45.45**			**81.81**			**52.52**	-

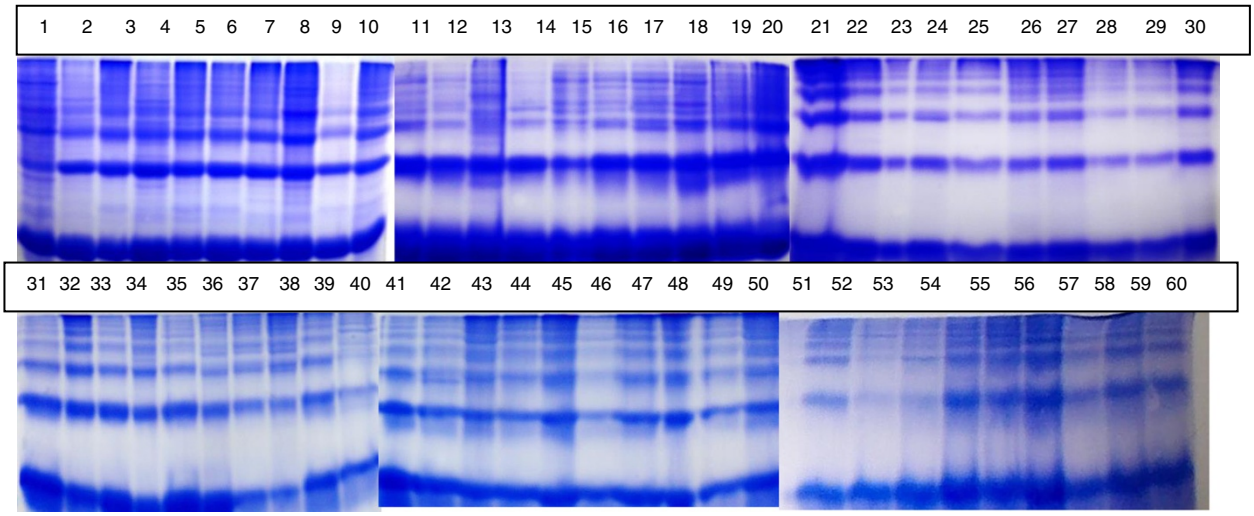

Figure 1 Comparison of patterns from of whole-cell protein profile of references strain *C. jejuni* ATCC 33291 (lane 1), and isolates from poultry carcass (lanes 2–60) on 12% SDS-PAGE.

increased surveillance and *Campylobacter* screening in food safety control to better protect consumers.

Figure 1 shows the protein profiles of the isolates as they appeared on the media of mCCDA and Preston agar compared with standard strains of *C. jejuni* ATCC 33291 on SDS-PAGE. The results demonstrated the presence of the common heavy protein band for 52 isolates compared with *C. jejuni* ATCC 33291. The whole-cell protein profiles of isolates were closely related to *C. jejuni* ATCC 33291. Some isolates could not be analyzed because they did not yield sufficient proteins after extraction, or the band did not resemble *C. jejuni* ATCC 33291. Any isolates showing discrepancy with the protein profile were excluded from confirmation of identification by using API CAMPY. Massai et al. (2007) explained that the whole-cell protein profile determined by SDS-PAGE expresses an important proportion of the genome. However, this expression may be modified by various factors. Therefore, this technique must be carefully controlled and standardized to obtain reproducible results. Advantages of the SDS-PAGE patterns are that they can be obtained in a short time, are reproducible, and do not require any sophisticated and expensive reagents or equipment compared with other molecular biology.

The polyacrylamide gel electrophoresis (PAGE) of bacterial proteins is an efficient technique for the classification of microorganisms, based on phenotypical characteristics expressed by their protein profiles (Scarcelli et al. 2001). Protein electrophoretic analysis of *Campylobacter spp*. in a polyacrylamide gel in the presence of SDS supplies data that can be used in epidemiological and taxonomic studies, as well as the identification of species and specific virulence factors (Dunn et al. 1987).

API campy strip system

After 24 to 48 h of incubation, the positive isolates obtained from SDS-PAGE were confirmed using API Campy, with 52 of 99 samples (52.52%) testing positive for both the first part and second part of the tests. Eleven of 99 (11.11%) isolates were negative. Forty-three of the 52 (82.69%) positive isolates were identified in both parts of the tests as *C. jejuni subsp. jejuni* 2. Five isolates were identified as *C. jejuni subsp. jejuni* 1 (9.61%), and 4 isolates were identified as *Campylobacter jejuni subsp. doylei* (7.96%). The reference strains were correctly identified by both systems as *C. jejuni ssp. jejuni* 1. Eleven isolates yielded discrepant identifications, and yielded profile codes absent in the API Campy database; therefore, they were considered negative results. Huysmans and Turnidge (1997) indicated that the correlation between API Campy and the conventional tests was 100% for the identification of *C. jejuni*.

Conclusion

The results show that *Campylobacter jejuni* was prevalent in poultry meat samples collected from wholesale markets in Riyadh City, Saudi Arabia. *C. jejuni* positive samples increased rapidly during storage at 4°C for approximately 1 wk. Our results also indicated a connection between the protein profiles on SDS-PAGE and API Campy used for the identification of *C. jejuni*.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Conceived and designed the experiments: YHM and AMM. Performed the experiments: YHM . Analyzed the data: YHM and AMM. Wrote the manuscript: YHM and AMM. All authors read and approve the final manuscript.

Author details
[1]Food Science and Nutrition Department, College of Food and Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia. [2]Food Science and Nutrition Department, Faculty of Home Economics, Helwan University, Cairo, Egypt.

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