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Prevalence and Antimicrobial Susceptibility of *Campylobacter* spp. in Live and Dressed Chicken in Jordan

Tareq M. Osaili,[1] Akram R. Alaboudi,[2] and Rani R. Al-Akhras[2,3]

Abstract

A total of 140 broiler flocks presented for slaughtering at Amman slaughterhouse were tested for *Campylobacter* spp. via collection of cloacal swabs from live birds, feathered skin samples at prescalding, and skin samples at postscalding (62°C or 57°C scalding temperature), postevisceration, and postchilling. The results indicated that 40% of the flocks tested by cloacal swabs, 34% at prescalding, 32% at post 57°C scalding, and 32% post-evisceration were harboring *Campylobacter jejuni*. None of the skin samples collected from dressed birds at postscalding (62°C) or postwashing-chilling steps (regardless of scalding temperature) revealed the presence of *C. jejuni*. Thirty eight isolates were tested for susceptibility to ten antimicrobials by using the microbroth dilution method. Almost 50% of the isolates were multidrug resistant to 9 or 10 out of the ten tested antimicrobials. The other half of tested isolates were sensitive to erythromycin, tetracycline, doxycyclin, chlortetracycline, ciprofloxacin, enorfloxacin, gentamycin, tilmicosin, amoxicillin, and trimethoprim.

Introduction

Campylobacter are the primary cause of bacterial foodborne gastroenteritis worldwide (Humphrey *et al.*, 2007). *Campylobacter jejuni* and *Campylobacter coli* are implicated in about 90% and 10% of the cases of human campylobacteriosis, respectively (Friedman *et al.*, 2000). *Campylobacter* spp. are frequently found in the intestine of wild and domesticated animals, especially birds (Jore *et al.*, 2010). Most *Campylobacter* infections are associated with cross contamination from raw chicken or consumption of undercooked chicken meat (Corry and Atabay, 2001; Humphrey *et al.*, 2007; Skjøt-Rasmussen *et al.*, 2009).

In recent publications, wide prevalence rates (6.3% to 91%) of *Campylobacter* in chicken (before and after slaughtering) have been reported worldwide (Moran *et al.*, 2009; Pepe *et al.*, 2009; Deckert *et al.*, 2010; Bardoň *et al.*, 2011). The contamination site of *Campylobacter* starts at the farm level where *Campylobacter* exists widely in the farm environment. At slaughter, contamination of broiler carcasses may happen at scalding, evisceration, or water chilling, and *Campylobacter* may persist in the product to the retail level (Stern and Robach, 2003).

The isolation of antimicrobial resistant *Campylobacter* strains from humans and animals has increased the concern of human *Campylobacter* infection. Prevalence of antimicrobial-resistant *Campylobacter* strains results from antimicrobials misuse in animal and poultry production for therapy and disease prevention (Deckert *et al.*, 2010; Bardoň *et al.*, 2011). Thus, it is important to monitor antimicrobial resistance in *Campylobacter* spp. of poultry receiving antimicrobial agents during rearing.

Although chicken meat is widely consumed in Jordan (22 kg/year/capita) (DS, 2009), no national criteria exist for routine *Campylobacter* monitoring plan in live and addressed chicken. In addition, no studies exist on the prevalence of *Campylobacter* spp. in chicken in Jordan. Therefore, this study aimed at determining the prevalence rate of *Campylobacter* spp. in live chicken in Jordan, the effect of abattoir processing on prevalence rate of *Campylobacter* spp. in dressed chicken, and antimicrobial profile of the isolated strains. This study will help in establishing a risk-based analysis system for *Campylobacter* and developing criteria for routine microbiological monitoring plan in Jordan.

Materials and Methods

Sample collection

Sample size was statistically determined by using a prevalence rate of 40%. Accordingly, one hundred forty broiler

[1]Department of Nutrition and Food Technology, Faculty of Agriculture, Jordan University of Science and Technology, Irbid, Jordan.
[2]Department of Pathology and Animal Health, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid, Jordan.
[3]Jordan Food and Drug Administration, Amman, Jordan.

chicken flocks (3000–5000 birds each) were randomly chosen during the study period (May–November/2009). Each flock was tested for the presence of *Campylobacter* spp. through cloacal swabs from fifteen live birds, after being hanged on a processing line at the Amman abattoir (Amman city). Amman abattoir is chosen in this study, because it is the only governmental slaughter house in Jordan and serves broiler farms from all over the country. In Amman, abattoir birds are manually slaughtered and scalded by immersion birds in an agitated single hot water tank using two temperatures: 57°C or 62°C for 90 sec at a rate of 86 birds/min. Evisceration is conducted by using a semi-automated system. A cut around the vent is mechanically done; however, in most times, viscera were manually withdrawn. Eviscerated carcasses are water bath washed (internally and externally) at an ambient temperature for 10 min with a recycling rate of 1 m^3/h. Chilling is performed by using counter flow current of constantly renewed ozonized (free ozone concentration 2.5 ppm) cold water (4°C) with a flow rate of approximately 2 L/carcass for 10 min (recycling rate of 1 m^3/h). Birds are mechanically propelled and are, thus, in a state of continuous agitation. This primary chilling step is followed by air chilling to a temperature of ≤2°C.

In the current study, the flocks were followed inside the slaughterhouse, and samples were collected from different processing steps: feathered skin samples at prescalding and skin samples at postscalding; postevisceration; and postchilling steps. Feathered skin samples and defeathered skin samples (*ca* 5 g each) were collected from pools of three sampling sites (feathered skin samples: neck, wings, and tail; defeathered skin samples: neck, chest, and around the evisceration opening). At each sampling point (postscalding; postevisceration; and postchilling), 25 randomly selected birds (forming 5 composite samples) from each flock were examined.

Isolation of Campylobacter *spp.*

The method described by ISO (2006) was followed for detection of *Campylobacter* spp. Skin and feathers samples were collected aseptically and homogenized through stomaching with Bolton broth (225 mL; Oxoid). All homogenates were incubated under microaerobic conditions at 37°C for 4 h and then at 41.5°C for 48 h. Inoculum (loopful) from homogenates was streaked onto modified charcoal cefoperazone deoxycholate (mCCD) agar (Oxoid), and microaerobically incubated at 41.5°C for 48 h. Cloacal swabs were directly inoculated onto mCCD agar and microaerobically incubated at 41.5°C for 48–72 h. All microaerobic conditions were conducted by using an anaerobic jar supplied with a gas generating kit (Campygen sachets, Oxoid).

The mCCD agar plates were inspected after incubation for the presence of colonies with typical *Campylobacter* morphology. The presumed *Campylobacter* colonies were subcultured onto the nonselective Columbia blood agar (Oxoid).

Identification of Campylobacter *spp.*

Campylobacter spp. were identified by Gram staining, motility test, oxidase test, catalase test, microaerobic growth at 25°C, aerobic growth at 41.5°C, Dryspot agglutination test, and biochemically by Hippurate hydrolysis test.

DNA extraction and polymerase chain reaction conditions

The genomic DNA was extracted from bacterial isolates by using Promega Wizard® Genomic DNA Purification Kit (Promega) following the manufacturer's instructions. *C. jejuni* specific primers for the polymerase chain reaction (PCR) assay were selected based on the published nucleotide sequence of a putative oxidoreductase subunit in the *C. jejuni* genome (Nayak *et al.*, 2005). The pair of primers F (5′-CAA ATA AAG TTA GAG GTA GAA TGT3′), R (5′- GGA TAA GCA CTA GCT AGC TGA T-3′) (Alpha DNA) were used to amplify a 160 bp DNA segment. The method as described by Nayak *et al.* (2005) was followed for amplification of the DNA fragment. All amplifications were performed on a thermo-cycler Veriti (Applied Biosystems). The amplified PCR products were examined by electrophoreses, visualized with a UV transilluminator, and photographed with the gel documentation system (Gel Doc 2000, Bio-Rad).

Susceptibility to antimicrobial agents

Representative number of *C. jejuni* strains isolates from live ($n = 19$) and dressed chickens ($n = 19$) were tested for susceptibility to antimicrobials. The thirty eight *C. jejuni* strains isolates in addition to the reference bacterial strain *C. jejuni* (ATCC 33291) were maintained on mCCD agar at 4°C and transferred biweekly to maintain viability.

The minimal inhibitory concentration (MIC) of 10 antimicrobials was determined by the microbroth dilution method described by Lalitha (2008). The antimicrobials examined were representative of those commonly licensed to be used in poultry production in Jordan. and included erythromycin-SCN, gentamycin sulphate, Norfloxacin, ciprofloxacin, doxycyclin HCl, tetracycline HCl (Tocelo Chemicals), trimethoprim (Biochem), and amoxicillin $3H_2O$ (Oman Biochem. and Pharmecuticals L.L.C.). Susceptibility profiles were determined by comparing calculated MIC values to available published breakpoints (Soussy *et al.*, 1994; US-FDA, 2004) for each tested antimicrobial.

Results

Prevalence of Campylobacter *spp. in tested flocks using conventional bacteriological method*

A total of 218 presumptively identified isolates were subjected to further confirmatory tests. All isolates were motile, curved, or spiral Gram negative and oxidase positive. A total of 134 isolates did not microaerobically grow at 25°C nor aerobically at 41.5°C and were positive for Dryspot agglutination test. Thus, the total number of *Campylobacter* spp. isolated from live and dressed chicken was 134 isolates. All these 134 isolates were positive for both Hippurate hydrolysis test and catalase test and, therefore, were identified as *C. jejuni*.

Fifty six and 48 out of 140 flocks sampled by either cloacal swabs and feathered skin, respectively, were positive for the presence of *C. jejuni.* No *C. jejuni* were detected in skin samples collected from 115 flocks skin samples scalded at 62°C, whereas 8 flocks out of the other 25 flocks tested after scalding at 57°C were positive for *C. jejuni.* Only 12% of flocks were positive after evisceration, but none of the tested samples from the 140 studied flocks revealed any isolates after washing and chilling step (Table 1).

Table 1. Prevalence of *Campylobacter jejuni* in Tested Flocks Before And During Processing Using The International Organization for Standardization (2006) Method

Sampling point	*Number of tested flocks*	*Number of flocks positive (%) for* Campylobacter jejuni
Cloacal swabs	140	56 (40)
Feathered skin, prescalding	140	48 (34)
Skin after scalding at 62°C	115	0 (0)
Skin after scalding at 57°C	25	8 (32)
Skin after evisceration (Scalding at 62°C)	115	14 (12)
Skin after evisceration (Scalding at 57°C)	25	8 (32)
Skin after washing-chilling	140	0 (0)
Total number of isolates		134

Prevalence of confirmed C. jejuni *in tested flocks using PCR technique*

The PCR gene analysis output using the 160 bp segment of oxidoreductase gene confirmed that all the 134 Dryspot, Hippurate hydrolysis, and catalase positive isolates are *C. jejuni.*

Susceptibility to antimicrobial agents

The MIC was defined as the lowest antimicrobial concentration that shows no growth of *C. jejuni* by visible reading. The MICs and sensitivity of selected *C. jejuni* isolates to 10 antimicrobial agents are presented in Table 2.

Reference strain (ATCC 33291) was sensitive to all 10 tested antimicrobials. Almost 50% of the isolates exhibited resistance to the tested fluoroquinolones (ciprofloxacin and enrofloxacin). Twenty one isolates (55%) revealed multiple drugs that were resistant and the other 17 isolates were totally sensitive to tested antimicrobials. Out of 21 multi-resistant isolates, 2 isolates showed resistance to only 2 antimicrobials (Amoxicillin and Trimethoprim), 3 isolates were resistant to 9 antimicrobials (2 isolates were sensitive to Tilmicosin, and 1 isolate was sensitive to Trimethoprim), and the other 16 isolates were totally resistant to all ten tested drugs.

Discussion

A prevalence rate of 40% for *C. jejuni* in live chickens (cloacal swabs) in the tested flocks comes in agreement with other studies which found that the prevalence range of *Campylobacter* spp. in broiler flocks (smear or cecal samples) was 41%–45% (Atanassova and Ring, 1999; Hein *et al.*, 2003). Chicken skin and feathers are an important reservoir for *C. jejuni*, and the feather is considered a major source of contamination in poultry processing plants (Keener *et al.*, 2004). In the current study, the prevalence of *C. jejuni* in the feathered skin samples collected at prescalding step (34%) was lower than that (92%) reported by Son *et al.* (2007) in the United States for samples collected from the prescalding step.

C. jejuni was detected from skin samples collected postscalding in many international articles. Good examples are the studies of Son *et al.* (2007) and Granic *et al.* (2009), where 90% and 85%, respectively, of skin samples examined postscalding were *Campylobacter* positive. In our study, *C. jejuni* were detected from 32% of flocks examined after scalding at 57°C for 90 sec, but not after scalding at 62°C for 90 sec, which is the temperature of scalding in routine work in Amman slaughterhouse. *Campylobacter* is generally heat sensitive ($D60 = 0.2–0.3$ min) (Keener *et al.*, 2004), and that may justify the absence of the organisms in our samples after scalding at 62°C for 90 sec. However, carcasses' surfaces were again recontaminated after viscera removal but at a quite low level (12%). This should be expected due to probable fecal content leakage which is quite common in poultry abattoir as has been clearly stated by other researchers that bird carcasses are rapidly contaminated by *Campylobacter* from bird's ceca during processing at abattoir (Miwa *et al.*, 2003).

The current study shows that no *C. jejuni* was recovered from skin samples collected after passing the washing and primary chilling step. It is well known that the chilling process reduces both the *C. jejuni* counts and number of positive carcasses (Figueroa *et al.*, 2009). The results of the latter study were obtained from the chiller tank by using chlorinated water. In our study, the chilling water was supplemented with ozone (O3). Ozone is a strong broad spectrum

Table 2. Minimum Inhibitory Concentration (μg/ml) and Sensitivity of Selected *Campylobacter jejuni* Isolates to Antimicrobials Using the Microtiter Dilution Technique

Antimicrobial (break points)	*MIC range of the tested isolates*	*Number of sensitive isolates*	*Number of resistant strains*	*Resistance (%)*
Erythromycin (≤1–4)[a]	0.12–≥128	19	19	50
Gentamycin (≤4–8)	0.12–≥128	21	17	44.7
Tilmicosin (≤2–8)	0.05–≥128	21	17	44.7
Ciprofloxacin (≤1–>2	0.06–8	19	19	50
Enrofloxacin (≤1–>2)	0.06–16	19	19	50
Tetracycline (≤4–8)	0.06–128	19	19	50
Doxycyclin (≤4–>8)	0.06–64	19	19	50
Chlortetracycline (≤8–16)	0.06–64	19	19	50
Amoxicillin (≤8–≥16)	4–≥128	17	21	55
Trimethoprim (≤4–>8)	8–≥128	18	20	52.6

[a]Selected breakpoints (S-R).

S-R = sensitive-resistant, breaking points are according to the National Antimicrobial Resistance Monitoring System (US-FDA, 2004); Soussy *et al.* (1994).

antimicrobial agent that inactivates wide spectrum of organisms. In primary concentration 2500 ppm, ozone has the ability to reduce pathogens to a nonculturable level and extend the shelf life of treated food (Rodriguez-Romo and Yousef, 2005).

The general incidence of resistance in our *C. jejuni* isolates among the ten antimicrobials tested with the microtiter dilution method was quite high. Gentamycin and erythromycin resistance is usually low in *C. jejuni* (Deckert *et al.*, 2010; Bardoň *et al.*, 2011). The MIC values for tilmicosin were almost in parallel to the other tested macrolid (erythromycin). The resistance pattern reported here is in the line of antimicrobial resistance reported for *C. jejuni* in the European Union Member States (EFSA, 2010). European Food Safety Authority (EFSA, 2010) published that 20%–64% of *Campylobacter* isolates from animals or their meat including poultry were resistant to fluoroquinolone. Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP, 2009) presented a ciprofloxacin-resistant percentage of 56.5% among *Campylobacter* isolates from imported broiler meat. Ampicllin susceptibility appears in a conflicting pattern. High figures of 58% and 52% resistance were published from Czech or USA, respectively (Griggs *et al.*, 2009; Bardoň *et al.*, 2011). On the other side, only 10% resistance was found in Canada (Deckert *et al.*, 2010). *C. jejuni* has been regarded as endogenously resistant to trimethoprim (Gibreel and Sköld, 1998), which is usually incorporated as a selective agent in media commonly in use for *Campylobacter* spp. isolation (Bopp *et al.*, 1982).

Fifty percent of the isolates exhibit resistance to targeted tetracycline's (doxycyclin, tetracycline). This is not different from the incidence recorded in the United States for organic or conventionally grown chicken (Luangtongkum *et al.*, 2006), where >60% of the *C. jejuni* isolates are resistant to doxycyclin and tetracycline. Similar to our finding, DANMAP (2009) reported that 51.6% of *C. jejuni* isolates from imported broiler meat were resistant to tetracycline.

The high percentage (50%) multi-resistance among our isolates is in the same trend of commonly reported multi-drug resistance *C. jejuni*. The apparent conflicting data might be related to different antimicrobial usage policies; different types of antimicrobials generally in use in each country and different standards of values are used for comparison.

In conclusion, the prevalence of *C. jejuni* at all levels of broiler chicken production in Amman slaughterhouse was low, and *C. jejuni* was not recovered from chilled dressed chicken. Almost half of the tested isolates showed a multi-resistance pattern. It is necessary to follow strict hygienic conditions in all broiler chicken production steps to keep the occurrence of *Campylobacter* spp. as low as possible and to control the inappropriate use of antimicrobials in poultry farms.

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Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Tareq M. Osaili, Ph.D.
Department of Nutrition and Food Technology
Faculty of Agriculture
Jordan University of Science and Technology
Irbid 22110
Jordan

E-mail: tosaili@just.edu.jo