

## Presence and characterization of *Campylobacter jejuni* in organically raised chickens in Quebec

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### Abstract

The objective of this study was to estimate the presence of the important foodborne pathogen *Campylobacter jejuni* in organically raised chickens in the province of Quebec. The recovered isolates were further characterized for their antimicrobial resistance profile, autoagglutination property and chemotaxis. Antimicrobial resistance was evaluated using agar dilution for: tetracycline, erythromycin, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, clindamycin, ampicillin, azithromycin, bacitracin, and ceftiofur. Autoagglutination was measured by monitoring optical density changes in a bacterial suspension after 3 h of incubation at room temperature. Chemotaxis was evaluated after a contact time of 3 h between isolates and mucin, using a quantitative protocol. A total of 10 lots of chickens was sampled in August and September 2009; half of them were positive for the presence of *C. jejuni*. Antimicrobial resistance was found only for tetracycline (44%), erythromycin (6%), azithromycin (6%) and clindamycin (2%). Variation was observed in the minimum inhibitory concentrations (MICs) for ceftiofur and bacitracin, for which *C. jejuni* possess intrinsic resistance. Autoagglutination and chemotaxis varied among isolates and lot-level differences in these were observed. Autoagglutination and chemotaxis levels appeared as independent isolate properties. Further monitoring and characterization of isolates originating from organic chickens is of interest since this type of production might represent another source of exposure of consumers to a variety of the foodborne pathogen *C. jejuni*.

### Résumé

L'objectif de cette étude était d'estimer la présence de *Campylobacter jejuni*, un agent pathogène alimentaire important, dans les élevages de poulets certifiés biologiques dans la province de Québec. Les isolats recueillis ont été caractérisés quant à leur profil de résistance aux antibiotiques, leur pouvoir d'auto-agglutination et de chimiotactisme. La résistance aux antibiotiques a été évaluée pour la tétracycline, l'érythromycine, le chloramphénicol, la ciprofloxacine, la gentamicine, l'acide nalidixique, la clindamycine, l'ampicilline, l'azithromycine, la bacitracine et le ceftiofur. L'auto-agglutination a été mesurée en effectuant le suivi de la densité optique d'une suspension bactérienne après 3 h d'incubation à température pièce. Le chimiotactisme a été évalué après un temps de contact de 3 h entre les isolats et de la mucine, en utilisant un protocole quantitatif. Un total de 10 lots de poulets a été échantillonné à l'abattoir en août et septembre 2009; la moitié des lots étaient positifs pour la présence de *C. jejuni*. Un phénotype de résistance aux antibiotiques a été déterminé pour la tétracycline (44 %), l'érythromycine (6 %), l'azithromycine (6 %) et la clindamycine (2 %). Une variation dans la concentration minimale inhibitrice (CMI) d'antibiotiques pour lesquels *C. jejuni* est naturellement résistant, la bacitracine et le ceftiofur, a été observée. L'auto-agglutination et le chimiotactisme étaient variables entre les isolats et des différences entre les isolats issus de différents lots ont été observées pour ces mêmes propriétés. L'auto-agglutination et le chimiotactisme semblaient non reliés. Une surveillance et une caractérisation accrue des isolats provenant des fermes certifiées biologiques est d'intérêt puisque ce type de production semble exposer le consommateur à une diversité de souches de *C. jejuni*.

(Traduit par les auteurs)

### Introduction

*Campylobacter jejuni* is recognized as a frequent bacterial foodborne agent in developed countries (1). Data on declared human cases of campylobacteriosis in 2009 in Quebec, Canada indicated a prevalence of 23.6 per 100 000 inhabitants, which has remained stable since the year 2000 (2). Risk factor studies (3) and investigations (4) of outbreaks of *C. jejuni* infections have identified consumption of chicken

and handling of raw chicken as important risk factors for campylobacteriosis. Similar conclusions have been reached by attribution studies comparing clinical and food isolates of *Campylobacter* (5) using different typing strategies (5,6).

A market is emerging for organically raised chickens due to a growing concern among consumers about bird welfare and use of antimicrobials on the farms (7). Some practices on organic farms, such as providing chickens with access to outdoor fields, probably

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expose birds to potential environmental sources of *Campylobacter* (8). As part of Quebec's organic reference standards (9), producers must provide chickens with access to the outside for at least 3 years before being eligible to obtain "organic" certification from the Conseil des appellations réservées et des termes valorisants (CARTV).

As observed in other studies, the status of organic chickens for *Campylobacter* is important because it has been shown that these chickens are colonized by *C. jejuni* (10–14). To our knowledge, no such studies so far have evaluated this topic in Quebec's organic production units. The importance of characterizing these isolates has been outlined in a recent study (15).

In the present study, isolate characterization was conducted by evaluating the following phenotypes: antimicrobial resistance, autoagglutination, and chemotaxis. Antimicrobial resistance is an important public health concern and is monitored in Canada by the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS). This monitoring program is already well-implemented in conventional chicken production, but not in organic chicken production. Assessment of antimicrobial resistance in organic production is of interest as it could provide information on the impact of suspending the use of antibiotics in conventional chicken production (16). In *C. jejuni*, autoagglutination has been demonstrated in isolates from chicken, as well as in biofilm formation and adhesion to, or invasion of, epithelial cells. It involves flagellin, mobility, and flagella glycosylation (17–20). Chemotaxis is also known as a factor in the virulence of *C. jejuni* (21,22). It is also associated with intestinal colonization in chickens (23) as well as with human infection (24). Identified attractants include amino acids, organic acids, chicken or bovine mucus, and constituents of mucus (mucins), which seem to favor chicken colonization (25,26).

As well as estimating the prevalence of *C. jejuni* in Quebec's organic chicken farms, this study obtained baseline information on the characterization of this biological hazard, in terms of its antimicrobial resistance, autoagglutination, and chemotaxis properties. The prevalence and characteristics of *C. jejuni* on Quebec's organic chicken farms are currently not known.

## Materials and methods

### Sampling and recovery of *Campylobacter jejuni*

Sampling took place in August and September 2009. The contact information of the producers asked to participate in the study was obtained from the CARTV Web site (9). The project was explained to producers in a telephone interview. During the sampling period, all available lots were sampled from those producers who agreed to participate. A lot was defined as a group of chickens originating from the same hatchery, at the same geographical address, raised during the same period of time and under the same conditions, and slaughtered on the same day. The birds were acquired from hatcheries that also supply conventional chicken farms. Half of the certified organic production units available at the time of the study participated (6 out of 12) and 10 production lots were sampled from those.

At the slaughterhouse, approximately 1 bird out of 10 was taken from each lot for sample analysis purposes 5 min after the slaughter

process began. Ceacum was taken from 30 chickens from each lot and transported on ice to the laboratory. For each lot, 3 samples of 10 g of fecal matter (1 g of fecal material per chicken) were constituted. The samples were mixed 1:1 (w/v) in buffered peptone water (AES Laboratory, Montreal, Quebec), directly plated on modified charcoal cefoperazone desoxycholate agar (mCCDA) Preston (Oxoid, Nepean, Ontario), and incubated at 42°C for 48 h in a microaerobic atmosphere using Oxoid's Atmosphere Generation System with the *Campylobacter* gas generation kit. Typical colonies were purified on mCCDA Preston (Oxoid) and then plated on blood agar (Fisher Scientific, Ottawa, Ontario). Each isolate was initially identified as *Campylobacter* based on motility under light microscopy (corkscrew motility) and Gram staining (spiral small gram-negative). Typical bacteria were further identified to the species level by polymerase chain reaction (PCR) as described in a previous report (27). Isolates that were confirmed as *C. jejuni* were then frozen in multiple aliquots at –80°C. For all negative samples by direct plating, samples were enriched in Bolton Broth (Oxoid) and *C. jejuni* was further isolated on mCCDA agar. All recovered isolates were used for further characterization.

### Antimicrobial resistance of *C. jejuni*

Antimicrobial resistance was determined by agar dilution using Mueller-Hinton agar plates (AES Laboratory) containing 5% (v/v) of defibrinated sheep blood (Oxoid), as defined by the Clinical and Laboratory Standards Institute (CLSI) (28). The following antimicrobial agents (Sigma-Aldrich, Oakville, Ontario) were tested: tetracycline (Tet), erythromycin (Ery), chloramphenicol (Chl), ciprofloxacin (Cip), gentamicin (Gen), nalidixic acid (Nal), clindamycin (Cli), ampicillin (AMP), azithromycin (Azi), bacitracin (Bac), and ceftiofur (Cef). These were chosen because they are considered important for treating human campylobacteriosis (Ery, Cip, Nal, and Azi) and because analog molecules are used in conventional poultry production (Tet, Chl, Gen, Cli, Bac, and Cef). Concentrations of antimicrobial agents tested ranged from 2 to 256 µg/mL. The minimum inhibitory concentration (MIC) breakpoints adopted were based on values used by CIPARS (29), with the exception of chloramphenicol and ampicillin, for which breakpoints were taken respectively from the 2006 report of the National Antimicrobial Resistance Monitoring System (NARMS) (30) and a report by the European Food Safety Agency (EFSA) (31). *Campylobacter jejuni* is naturally (or intrinsically) resistant to bacitracin and ceftiofur (32,33). No breakpoint exists for these resistances as no antimicrobial resistance study has included them in their antimicrobial panels; our analysis will therefore focus on the distribution of MICs.

Fresh bacterial cultures were suspended in phosphate-buffered saline (PBS) (Oxoid) in a concentration equivalent to 0.5 McFarland standards. Isolates were put down on Mueller-Hinton agar plates (AES Laboratory) containing 5% (v/v) of defibrinated sheep blood (Oxoid) and on the corresponding plates containing each of the tested antimicrobial concentrations. Agar plates were then incubated at 42°C in a microaerobic atmosphere for 24 h. For each of the recovered isolates, the MIC recorded corresponded to the lowest concentration of antimicrobial inhibiting the growth of the isolates. The *C. jejuni* sequenced strain RM1221 (34) (ATCC BAA-1062)

(Cedarlane Laboratories, Burlington, Ontario) and *E. coli* ATCC 25922 were used as control.

## Autoagglutination

Autoagglutination was assessed based on previous studies with some modifications (20). Isolates were taken from single  $-80^{\circ}\text{C}$  storage, plated on mCCDA Preston, and incubated at  $42^{\circ}\text{C}$  for 24 h in a microaerobic atmosphere. Isolates were then put on blood agar for another 24 h. Isolates were suspended in PBS to an optical density (OD) of 1.0, measured at 630 nm in a cuvette containing 1 mL of the initial suspension. Bacterial suspensions in this cuvette were maintained without disturbance at room temperature for 3 h; OD was then remeasured using the same cuvette. Autoagglutination was expressed as follows:

$$(\text{OD}_{\text{initial}} - \text{OD}_{3\text{h}}) / \text{OD}_{\text{initial}} \times 100$$

For each isolate, the autoagglutination experiment was produced in a technical triplicate. All isolates recovered from organic chickens were tested. Sequenced strains rm1221, nctc11168 (ATCC 700819, Cedarlane), and 81-116 and 81-176 were also tested as controls. Previous studies established that strains 81-176 (17), nctc11168 (35), and 81116 (36) were proven autoagglutinating.

## Chemotaxis

The chemotaxis assay was adapted by combining a cell culture assay (37) and a classical assay (26), creating a new experimental approach to evaluate this phenotype for *C. jejuni*. Fresh isolates were suspended in PBS to an OD of 1.0 (630 nm), as for autoagglutination. From this suspension, 200  $\mu\text{L}$  were centrifuged at  $3000 \times g$  for 10 min. The supernatant was then discarded, replaced with 0.125% (w/v) agar (BD, Mississauga, Ontario) in PBS, and the bacterial pellet was resuspended. The soft agar containing the bacteria was then poured into a transwell insert (BD) of 1- $\mu\text{m}$  pore size and maintained at  $4^{\circ}\text{C}$  for 5 min. The insert was placed in a 24-well microplate (BD) containing either PBS (negative control) or a 2 mg/mL bovine gastric mucin solution (Sigma-Aldrich) in PBS. The plates were incubated at  $42^{\circ}\text{C}$  for 3 h in a microaerobic atmosphere.

After incubation, the inserts were removed and the concentration of bacteria found in the mucins or PBS solution, as well as the concentration of the initial bacterial suspension, were determined by serial dilution and plating on Mueller-Hinton agar incubated at  $42^{\circ}\text{C}$  for 48 h in a microaerobic atmosphere. The chemotaxis potential, at 3 h, of each isolate was determined as a ratio (R) defined as the number of bacteria found in the mucins solution (B) with the number of bacteria contained in the negative PBS control (C) subtracted and divided by the number of bacteria initially contained in the soft agar transwell insert (A). The chemotaxis ratio is therefore expressed as:

$$R = (B - C) / A$$

To facilitate graphical representation and statistical analysis, this ratio was transformed as follows:

$$1 / -\log(R)$$

Each chemotaxis isolate experiment was conducted in a technical triplicate. All isolates recovered from organic chickens were tested, as

well as the following strains: rm1221, nctc11168, 81-116, and 81-176. Chemotaxis of strains nctc11168 and 81-176 was previously described (21–23,26). Only strain nctc11168 had previously been shown to be attracted by mucin (26).

## Phenotyping

To qualify the overall phenotype similarity of the isolates (anti-microbial resistance profile, autoagglutination, and chemotaxis), a dendrogram (Bionumerics 6.1; Applied Maths, Saint-Martens-Latem, Belgium) was generated using phenotypic values for each isolate (expressed as dichotomic or quantitative as presented in the legend for Figure 5) and using Rank correlation similarity coefficient and complete linkage for clustering.

## Statistical analysis

All statistics were calculated using the NCSS/Statistical & Power Analysis Software (PASS) with an alpha level of 0.05. The chi-squared test was used to evaluate the link between antimicrobial resistance and the lot origin of the isolates. The Kruskal-Wallis test was used to compare autoagglutination or chemotaxis among the lots, the Fisher exact test was used to compare pairs of lots with different phenotypes, and the Kolmogorov *t*-test was used to compare the mean chemotaxis between the 2 autoagglutination groups.

# Results

## Recovery of *C. jejuni*

A total of 54 *C. jejuni* isolates was successfully recovered from the sampled lots from a total of 10 lots. The lots came from 6 organic chicken producers (out of 12 available on the CARTV web site) and were located in 4 of Quebec's administrative regions. All negative pooled samples by direct plating were also negative when using enrichment broth. *C. jejuni* was isolated in 50% of the lots [95% confidence interval (CI), 19-81].

## Antimicrobial resistance

It was found that some *C. jejuni* isolates were resistant to tetracycline, erythromycin, azithromycin, and clindamycin (Table I). One isolate was resistant to 3 antimicrobial agents (azithromycin, clindamycin, and erythromycin). The MIC distribution for bacitracin appears bimodal, with concentrations of either 64  $\mu\text{g}/\text{mL}$  or 256  $\mu\text{g}/\text{mL}$  and higher. For ceftiofur, the MICs of isolates were found to be centered on 64  $\mu\text{g}/\text{mL}$ . The presence of resistance to tetracycline in isolates was found to vary depending on the origin of the lot ( $P < 0.0001$ ). Lots 1 and 5 presented a high proportion of isolates that were resistant to tetracycline, which was the opposite of Lot 2 ( $P < 0.0001$ ).

## Autoagglutination

Autoagglutination varied depending on the different isolates (Figure 1). Graphical analysis of the distribution of autoagglutination values of the isolates after 3 h suggests that there are 2 defined groups. In the first group, 21 out of 54 isolates (39%) could be considered as autoagglutinating and the recorded values varied from 42%

**Table I. Distribution of minimum inhibitory concentration (MIC) and resistance of *C. jejuni* isolated from organic chicken lots**

Antibiotics	Breakpoint ( $\mu\text{g/mL}$ )	MIC ( $\mu\text{g/mL}$ ) distribution (%)										Isolate resistance (%)
		< 2	2	4	8	16	32	64	128	256	> 256	
Tetracycline	$\geq 16$	43	0	0	14	2	27	4	10	0	0	44
Erythromycin	$\geq 32$	84	0	11	0	0	0	0	0	0	5	6
Chloramphenicol	$\geq 32$	64	0	27	9	0	0	0	0	0	0	0
Ciprofloxacin	$\geq 4$	100	0	0	0	0	0	0	0	0	0	0
Gentamicin	$\geq 8$	100	0	0	0	0	0	0	0	0	0	0
Nalidixic acid	$\geq 64$	0	2	70	5	23	0	0	0	0	0	0
Clindamycin	> 4	95	0	4	1	0	0	0	0	0	0	2
Ampicillin	> 16	3	0	61	32	4	0	0	0	0	0	0
Azithromycin	$\geq 8$	95	0	0	0	0	0	0	5	0	0	6
Bacitracin	Undefined	0	0	0	0	0	0	31	5	30	34	nd
Ceftiofur	Undefined	0	0	0	0	0	4	59	28	2	7	nd

N — 54 isolates; nd — not determined.

to 90%. On the other hand, a high proportion of isolates (23 out of 54) showed the opposite phenotype, with values not higher than 8%.

Considering this distribution, the autoagglutination values were investigated according to lot origin (Figure 2). Lots were found that had different isolate autoagglutination ( $P < 0.0001$ ). Two lots presented opposite profiles: 1 with exclusively low autoagglutination (Lot 1,  $n = 19$ ) and the other (Lot 2,  $n = 16$ ) with all isolates presenting high values (mean 78%). The 3 other lots presented intra-lot variability of autoagglutination with high mean values and large confidence intervals (CI). Isolates from Lot 1 had a lower autoagglutination mean than all other lots (versus Lot 2,  $P < 0.0001$ ; versus Lot 3,  $P = 0.0011$ ; versus Lot 4,  $P = 0.0098$ ; and versus Lot 5,  $P = 0.0022$ ). Autoagglutination was as follows for the 81-176, 81116, nctc11168, and rm1221 strains:  $50.37 \pm 0.26$ ,  $48.79 \pm 1.21$ ,  $74.67 \pm 0.56$ , and  $51.43 \pm 7.6$ , respectively.

## Chemotaxis

The chemotaxis distribution of *C. jejuni* showed a variation between a minimum value of 0 and a maximum of 1.1 (Figure 3). No distinct groups seemed to emerge based on the distribution analysis. Chemotaxis was also investigated according to the lot of origin (Figure 4). It was found that lots had different isolate chemotaxis ( $P = 0.0314$ ). Isolates from Lot 3 and Lot 5 presented a higher attraction mean toward mucin than Lot 2 ( $P = 0.006$  and  $P = 0.0287$ , respectively), which presented isolates with lower chemotaxis. The protocol used allowed for a quantitative characterization of the isolates regarding chemotaxis. Chemotaxis of strains rm1221, nctc11168, 81-116, and 81-176 were  $0.14 \pm 0.12$ ,  $0.14 \pm 0.12$ ,  $0.20 \pm 0.17$ , and  $0.46 \pm 0.18$  respectively.

Based on the groups defined by autoagglutination, mean chemotaxis of isolates from group A (autoagglutination < 40%) and group B (autoagglutination > 40%) were  $0.26 \pm 0.24$  and  $0.19 \pm 0.14$ , respectively. The 2 means were not found to be different ( $t$ -test, Kolmogorov,  $P > 0.05$ ).

## Dendrogram analysis

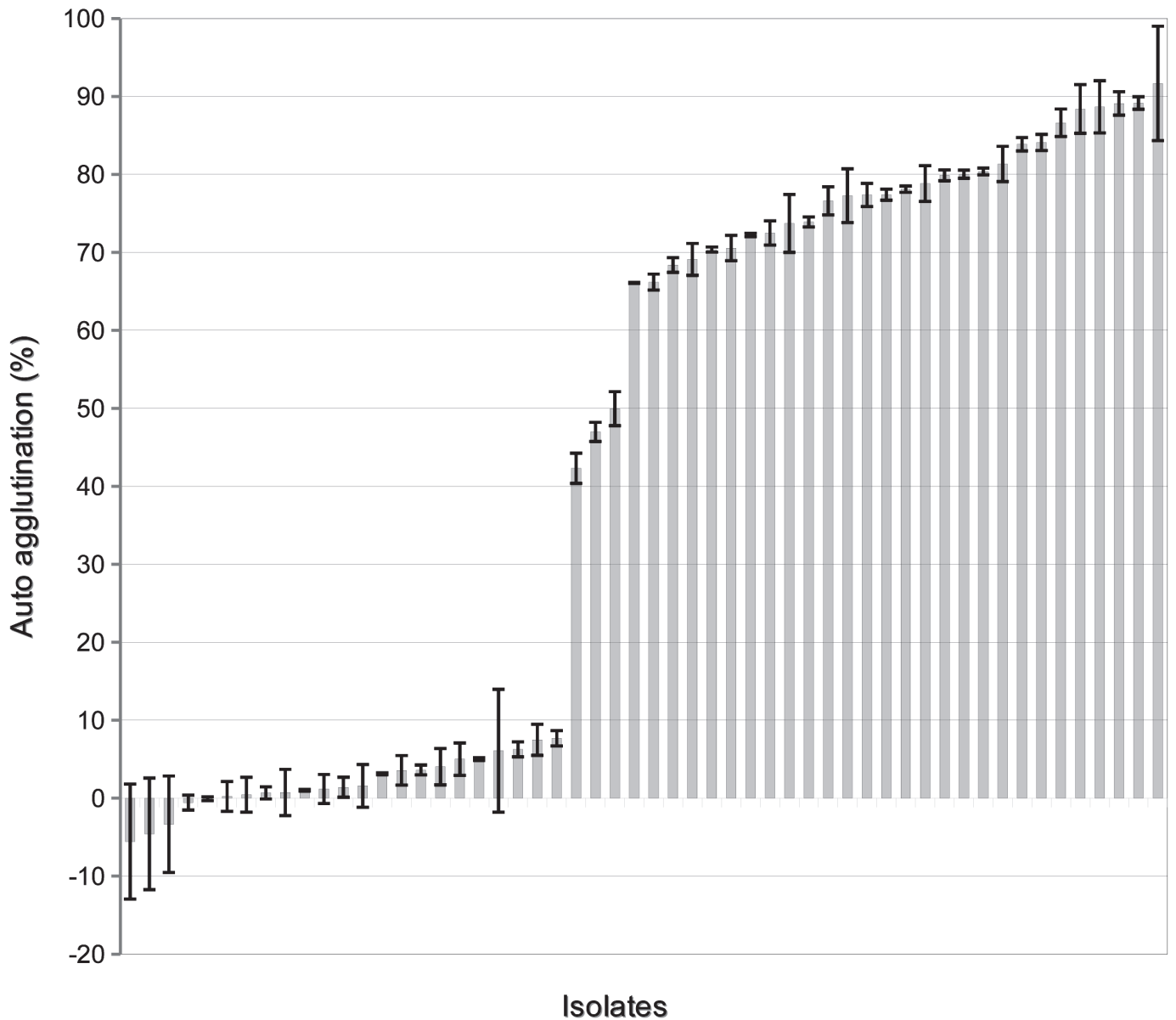
The lots seemed to be composed of isolates that presented different antimicrobial resistance profiles, autoagglutination, and

chemotaxis. In this study, these phenotypes were associated to some degree with the lot origin of the isolate. By gathering phenotypical information, it might be possible to evaluate the similarity of phenotypes among the isolates and to test the question of the variety of isolates according to the sampled lot of chickens. For this reason, a dendrogram was generated using the phenotypical data gathered (Figure 5).

At first glance, 2 large clusters were easily identified: 1 composed principally of tetracycline-resistant isolates with lower autoagglutination capacities and another composed of isolates sensitive to tetracycline and having high autoagglutination capabilities. Based on a graphical analysis, 8 clusters of isolates were further defined. Cluster 1 (3 isolates) was characterized by isolates with multiple antimicrobial resistances and high autoagglutination. Cluster 2 (5 isolates) regrouped isolates having low bacitracin MIC and high autoagglutination. Cluster 3 (16 isolates) was formed by isolates with higher bacitracin MIC and high autoagglutination. Cluster 4 (3 isolates) was made up of isolates showing high bacitracin MIC, high autoagglutination, and low chemotaxis. For cluster 5 (11 isolates), it could be observed that the isolates possessed low bacitracin MIC and tetracycline resistance with moderate MICs. Cluster 6 (8 isolates) was similar to cluster 5, except that there was higher tetracycline resistance MIC and autoagglutination. Cluster 7 (3 isolates) regrouped isolates based on their high bacitracin MIC, no tetracycline resistance, low autoagglutination, and low chemotaxis. Finally, cluster 8 (5 isolates) presented isolates with equally low chemotaxis and moderate to low bacitracin and tetracycline MIC. Lots 1 to 5 contained the following number of clusters: 4, 3, 3, 1, and 2 respectively.

## Discussion

In this study, data were gathered that would allow us to assess the prevalence and characteristics of *C. jejuni* in organically raised chickens in Quebec. This is the first time, to our knowledge, that the prevalence, antibiotic resistance, and specific phenotypes for organic chicken production in Quebec have been reported.



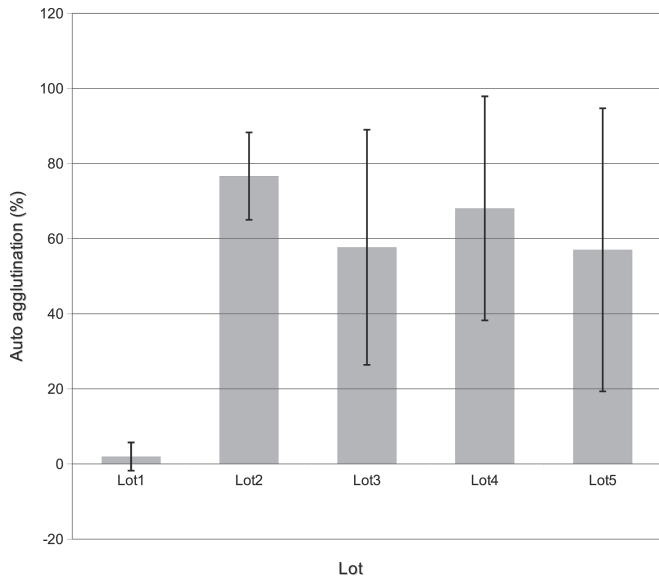
**Figure 1. Autoagglutination of *C. jejuni* isolated from organic chicken lots (N = 54 isolates), sorted by ascending values. Errors bars: standard deviation**

Previous studies have reported that the prevalence of *C. jejuni* can be as high as 100% in organic chicken lots (12). In this study, 50% of the lots sampled were positive. The prevalence of organic lots that tested positive to *C. jejuni* appeared to be higher in value than in Quebec in 2003 (50% versus 35%), when the ceecal prevalence of *C. jejuni* in conventional chicken lots was last reported (38). This presence suggests that consumption of organic chickens might increase the exposure of consumers in Quebec to *C. jejuni*. The presence of this foodborne pathogen in the gut of slaughtered animals is in fact associated with its presence on the carcasses (39). Moreover, isolates from free-range chickens (birds with access to an outdoor field) were found to be similar to those retrieved on retail meat and in cases of human campylobacteriosis (14). A recent study also found that isolates identified in organic chickens were similar before and after slaughter (15). In this study, the observed presence of the

pathogen at slaughter suggests that the end product might also be contaminated by *C. jejuni*. More studies are needed, however, to establish that isolates found in Quebec's organic chickens can be linked to human campylobacteriosis. The isolates were sampled at the slaughterhouse. Although it cannot be completely ruled out that the isolates were the result of contamination of the birds during transport, the level of caecal colonization observed strongly suggests that the contamination was acquired on the farm.

Previous studies of organic chicken production showed a low level of antimicrobial resistance isolated from organic or free-range production (10,12,16). Antimicrobial resistance in Canada is monitored each year by CIPARS. In 2009, CIPARS reported partial data on antimicrobial resistance, which showed that *C. jejuni* isolated from retail meat from conventional farms in Quebec were found to be resistant to azythromycin (4 isolates out of 48), clindamycin





**Figure 2. Mean autoagglutination value of *C. jejuni* isolates per organic lot. Isolates per lots (Lot 1, N = 19; Lot 2, N = 16; Lot 3, N = 10; Lot 4, N = 2; and Lot 5, N = 7). Errors bars: standard deviation**

(1 isolate out of 48), erythromycin (4 isolates out of 48), and tetracycline (30 isolates out of 48) (40). For these antimicrobials, with the exception of telithromycin, which was not covered in our study, and clindamycin for which the resistance level seems to be the same between the CIPARS and our study, resistance appeared slightly lower in values in the organic chicken isolates sampled in 2009 compared to the CIPARS data. This might be because organic farms in Quebec do not use antimicrobials for a minimum period of 3 y before certification. Antimicrobials used in broiler chickens can be selected for resistance to *C. jejuni* (41). If their use is stopped at the farm, the antimicrobial resistance of the associated bacteria is reduced (42,43). Isolates recovered from organic chicken farms also have lower antimicrobial resistance levels for *C. jejuni* than those from conventional farms (10,11).

In this study, tetracycline resistance was observed in *C. jejuni* even though this antimicrobial has not been used on organic chicken farms for at least 3 years. Tetracycline resistance was also reported in other studies of organic chickens, with prevalence ranging from low (0.05%) (12) to high (79%) (13). Even without antimicrobial pressure, some isolates in our study remained resistant to erythromycin, azithromycin, and clindamycin. Such resistance was also found in isolates originating from other organic productions (12,13,16). In *C. jejuni*, the same mechanism of resistance may be observed for clindamycin (lincosamine), erythromycin, and azythromycin (both macrolide). Resistance in *C. jejuni* can be caused by efflux or by modification of the antimicrobial target by punctual mutation of the genes, or by both (44).

The level of resistance to bacitracin and ceftiofur, antimicrobials for which no breakpoints are defined, was also investigated. A range of MICs (64 to over 256 µg/mL and 32 to over 256 µg/mL) was observed in this study for both these antimicrobials, respectively. These differences in susceptibility may indicate that the isolates harboured different mechanisms for resisting these antimicrobials.

Since it was found that some isolates were not sensitive to the highest concentration of bacitracin and ceftiofur used, in further studies, dilution series for these antimicrobials should go beyond concentrations of 256 µg/mL. This observation for intrinsic resistance was also previously reported for streptogramin in *Enterococcus* (45). Resistance to ceftiofur (β-lactame) is caused by a reduced affinity of *C. jejuni* porins to the antibiotic or low-level production of β-lactamase (33). The exact resistance mechanism to bacitracin for *C. jejuni* still needs to be clearly characterized (32). These antimicrobial susceptibilities could be continuously monitored in order to follow the evolution of the situation over time in the organic environment where no antimicrobial pressure is applied to the isolates.

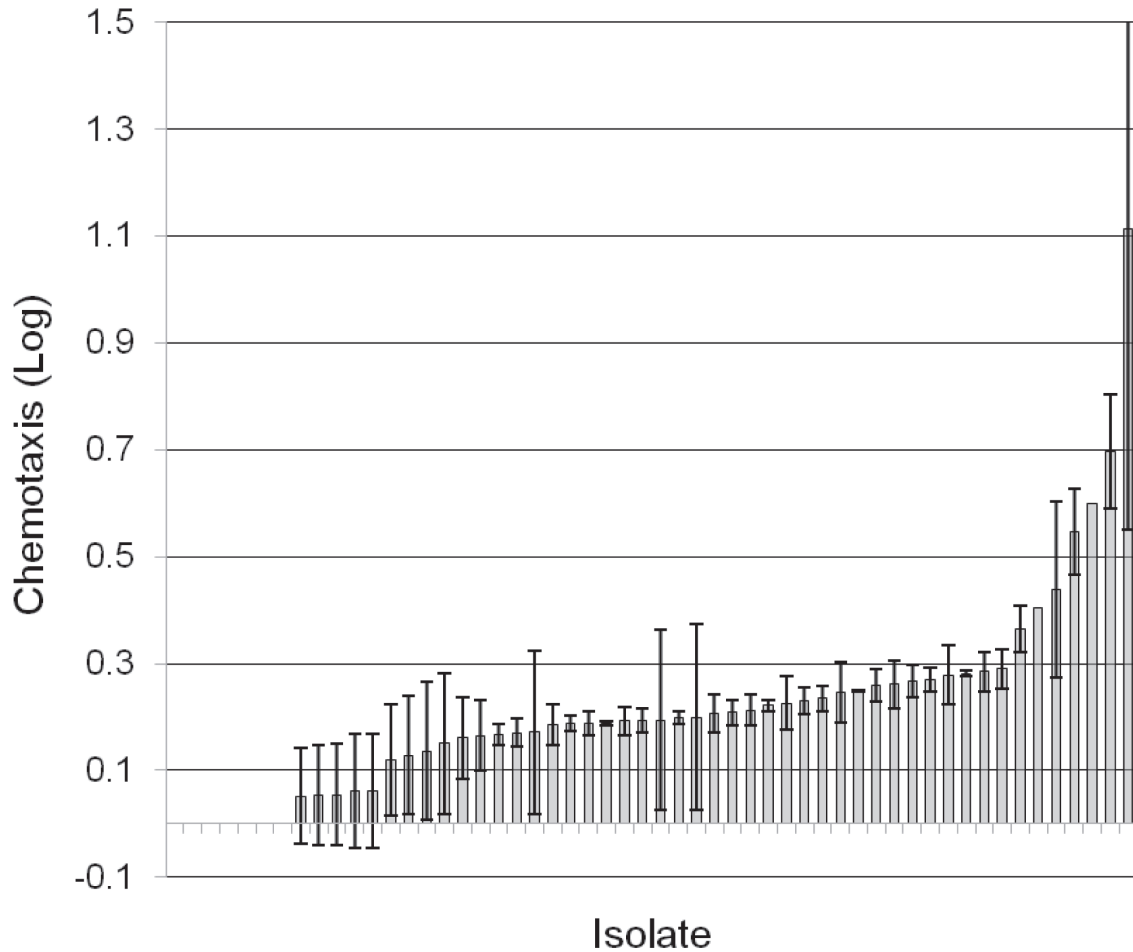
Autoagglutination is caused by interactions between bacteria that create aggregates. This phenotype is involved in the pathogenesis of enteropathogens such as *Yersinia* (46), *Enterococcus* (47), and *E. coli* (48). *Campylobacter* autoagglutination has previously been studied after a 24 h incubation period at room temperature (17,22,35). We chose to use a 3-h incubation, based on preliminary assays showing that agglutination was more variable in our isolate population at this time. Also, in this preliminary work, previously characterized strains (81-176, nctc1116, and 81116) remained positive for autoagglutination at 3 h, which shows that this timeframe allows autoagglutinating isolates to be detected.

Autoagglutination of the tested *C. jejuni* at 3 h showed variability between isolates and allowed for their differentiation. Flagellum is important for *C. jejuni* autoagglutination (17–19). The *C. jejuni* flagella apparatus varies considerably from isolate to isolate (49). The observed variability shown in the field isolates was expected, but the association of lots with this *Campylobacter* property is original and described for the first time.

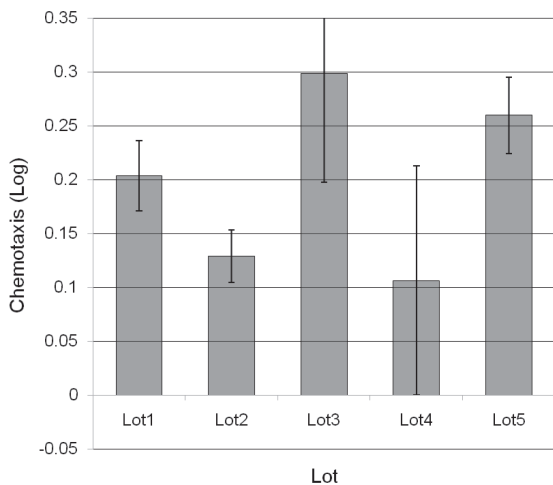
Chemotaxis is the ability of motile bacteria to move toward or against a concentration of a specific molecule. It is also an important virulence factor for different pathogenic bacteria such as *Salmonella* sp. (50). The protocol developed in this study is based on bacterial enumeration rather than a migration diameter, which is the protocol used in most studies (21,26). The method used in this study allowed the isolates to be classified according to their chemotaxis. Strains for which chemotaxis has previously been characterized (nctc11168 and 81-176) (21–23,26) were also confirmed positive in our assay. We also confirmed and quantified chemotaxis towards mucins for 2 other commercially available sequenced strains, rm1221 and 81116.

This approach to characterizing *C. jejuni* in terms of chemotaxis is of interest since it allows the phenotype to be quantified. The great variability observed among isolates in this study, ranging from 0 to 1.1 despite confirmation of motility under light microscopy, requires further study. The presence of the different chemotaxis mechanisms in the farm population of *C. jejuni* has not yet been assessed and should be determined since chemotaxis is a virulence factor for *C. jejuni* and could be used as a target to control the pathogen at the farm.

In the tested field isolates, chemotaxis was not associated with autoagglutination. Both autoagglutination groups had the same chemotaxis mean. This finding highlights that the 2 phenotypes are not necessarily related in field isolates, even though flagella and mobility are important for these 2 bacterial properties. It has been observed that isolates with mutation affecting autoagglutinating may remain



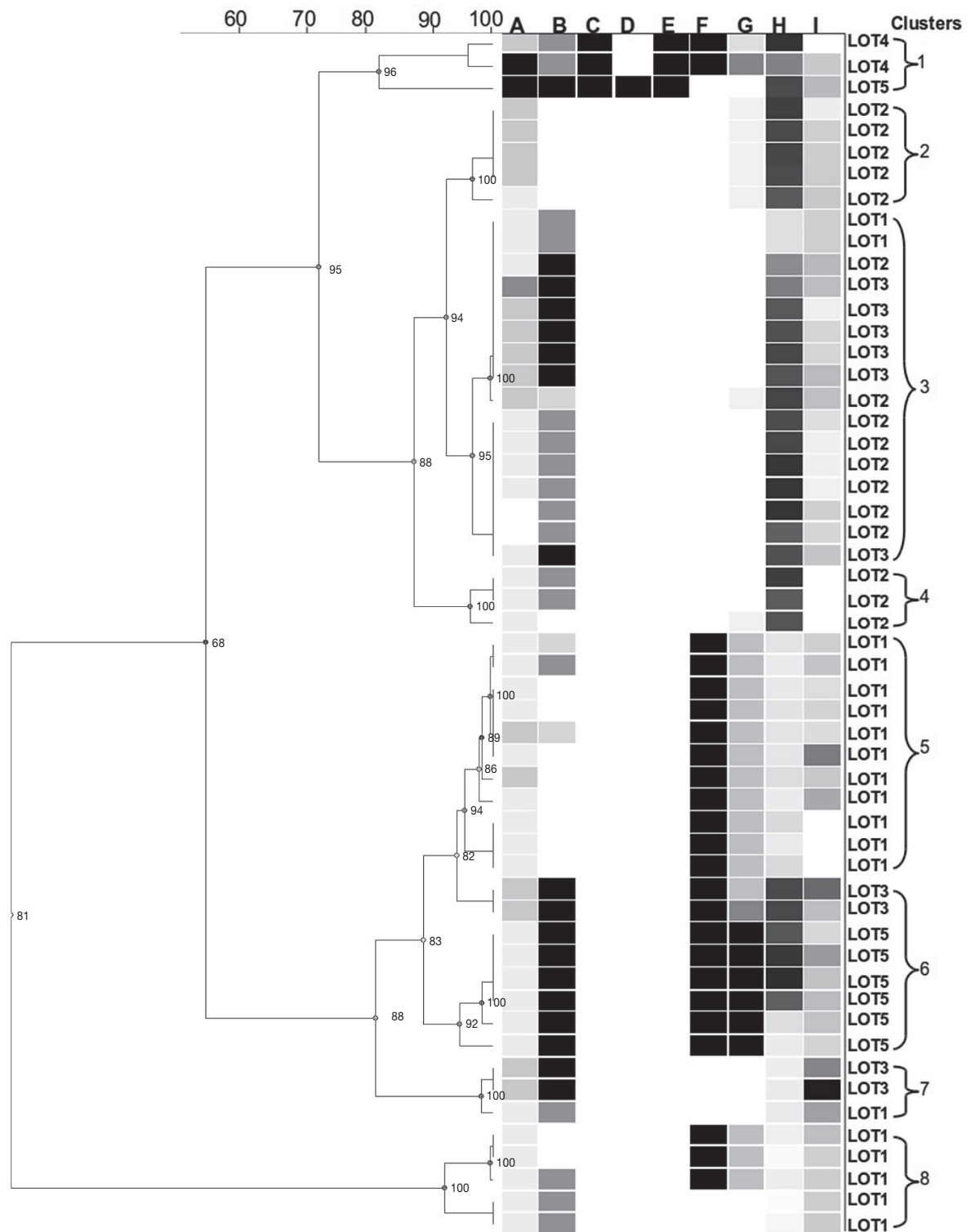
**Figure 3.** Chemotaxis of *C. jejuni* isolated from organic chicken lots (N = 54), sorted by ascending values. Errors bars: standard deviation



**Figure 4.** Mean chemotaxis values of *C. jejuni* isolated from organic chickens according to lots. Isolates per lots (Lot 1, N = 19; Lot 2, N = 16; Lot 3, N = 10; Lot 4, N = 2; and Lot 5, N = 7). Errors bars: standard deviation

mobile and vice versa (17,18,20). Other virulence mechanisms are probably present in our isolates that could influence their properties. Further phenotypic investigation as well as the identification of the gene contents of *C. jejuni* by microarray is of interest in order to better understand the relationship between *C. jejuni* and chickens. In our experimental conditions, evaluation of chemotaxis and autoagglutination of *C. jejuni* was found to be quite fastidious, confirming that these results are susceptible to variation of the environmental conditions of assays. Nevertheless, these first descriptive results on *C. jejuni* isolated from organic chickens in Quebec are of great interest from the perspective of future and complementary studies.

The observations of the present study make it possible to hypothesize that some practices used in organic chicken farming may select for specific phenotypes within a diverse population of *C. jejuni*. The phenotypes identified in this study allowed the isolates to be characterized inter and intra lot. It is worthwhile to monitor the evolution of the organic *C. jejuni* population. It would be interesting to observe whether the antimicrobial-resistant isolates remain or if the autoagglutinating group and the tetracycline-resistance group



**Figure 5. Phenotypal clustering of *C. jejuni* isolates recovered from organic chicken lots.**  
**A** — ceftiofur minimum inhibitory concentration (MIC).  
**B** — bacitracin MIC.  
**C** — azithromycin resistance.  
**D** — clindamycin resistance.  
**E** — erythromycin resistance.  
**F** — tetracycline resistance.  
**G** — tetracycline MIC.  
**H** — autoagglutination.  
**I** — chemotaxis.



expand in proportion. This study provides the basis for parameters that could be followed up in the future.

In conclusion, some organic chicken lots sampled in Quebec were positive for *C. jejuni*, which establishes this presence for the first time and suggests a possible contribution of these types of productions to human campylobacteriosis. More studies are needed to clearly establish this. Observed antimicrobial resistance was low (erythromycin 6%, clindamycin 2%, and azythromycin 6%), with the exception of tetracycline resistance, which was higher (44%). A variation of the MIC for ceftiofur and bacitracin, which are intrinsically resistant in *C. jejuni*, was observed. This suggests that these isolates have different mechanisms of resistance for these 2 antimicrobials. The other evaluated phenotypes, autoagglutination and chemotaxis, were variable. Opposite phenotypes were found in different lots. We also proposed a new way to evaluate chemotaxis, based on bacterial enumeration. Continuous monitoring and characterization of isolates from organically raised chickens in Quebec are of interest as this type of production represents another possible exposure route for consumers to a variety of the foodborne pathogen *C. jejuni*.

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