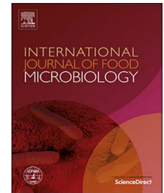
International Journal of Food Microbiology 253 (2017) 43–47

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro



Epidemiology of antimicrobial resistant *Campylobacter* spp. isolated from retail meats in Canada

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ARTICLE INFO

Keywords:
Campylobacter
Retail meat
Antimicrobial resistance
Comparative genomic fingerprinting

ABSTRACT

Campylobacter is an important zoonotic pathogen found in livestock and can cause illness in humans following consumption of raw and undercooked meat products. The objectives of this study were to determine the prevalence of *Campylobacter* spp. in retail meat (poultry, turkey, pork and beef) purchased in Alberta, Canada and to assess antimicrobial resistance and genetic relatedness of recovered *Campylobacter* strains with previously isolated strains from clinical and environmental sources. A Comparative Genomic Fingerprinting (CGF) method was used for assessing genetic relatedness of isolates. A total of 606 samples comprising 204, 110, 145 and 147 samples of retail chicken, turkey, ground beef and pork, respectively, were obtained. *Campylobacter* was isolated from 23.5% (48/204) of chicken samples and 14.2% (8/110) of turkey samples. Pork and beef samples were negative for *Campylobacter*. *Campylobacter jejuni* was the most common (94.6%) spp. found followed by *C. coli* (5.4%). Resistance to tetracycline was found in 48.1% of isolates, followed by resistance to ciprofloxacin (5.5%), nalidixic acid (5.5%), azithromycin (1.78%), and erythromycin (1.78%). All isolates were susceptible to clindamycin, florfenicol, gentamicin and telithromycin. Tetracycline resistance was attributable to the presence of the *tet*O gene. CGF analysis showed that *Campylobacter* isolated from poultry meat in this study were genetically related to clinical isolates recovered from human infections and to those isolated from animals and the environment.

1. Introduction

The National Enteric Surveillance Program (NESP) (PHAC, 2014) in Canada reported that *Campylobacter* isolates represented 11.8% of the enteric pathogens isolated from humans and was the top foodborne pathogen reported. Data from the U.S. suggested that campylobacteriosis is the second most prevalent cause of gastroenteritis and is responsible for a significant number of foodborne illnesses and deaths each year (CDC, 2016; Nyachuba, 2010). The majority of human infections are caused by *C. jejuni* with most of the remaining infections being attributed to *C. coli*. Most *Campylobacter* infections in humans are associated with the ingestion of contaminated raw/undercooked poultry products (EFSA, 2010; Suzuki and Yamamoto, 2009); however other types of meats, such as pork and ground beef have also been associated with *Campylobacter* species (Korsak et al., 2015; Trokhymchuk et al., 2014).

Although most *Campylobacter* infections are self-limiting and do not require treatment with antimicrobials, severe and prolonged cases of campylobacteriosis and infections in immuno-compromised, vulnerable populations and children may require antimicrobial therapy. In these instances, erythromycin and fluoroquinolone (*i.e.* ciprofloxacin) are the drugs of choice (Allos, 2001; Engberg et al., 2001). In addition to *Campylobacter* infection with the risk to develop long term sequelae, the development of resistance to antimicrobial drugs by *Campylobacter* strains is also a concern. Antimicrobial resistance in *C. jejuni* is increasing globally (Alfredson and Korolik, 2007; Bohaychuk et al., 2006; Kos et al., 2006a). Quinolone resistance is relatively common in *C. jejuni* (Iovine and Blaser, 2004) and resistance mostly arises from a mutation in the quinolone resistance determining region (QRDR) of the *gyrA* gene (Alfredson and Korolik, 2007). Occasionally, a mutation in the *parC* gene encoding for topoisomerase IV may also result in reduced susceptibility to quinolones. Another important antibiotic used for the treatment *Campylobacter* infections is erythromycin, a macrolide, whose resistance in *Campylobacter* is chromosomally mediated and mainly due to mutation in domain V of 23S rRNA (Lehtopolku et al., 2011). Generally, tetracycline is rarely used to treat campylobacteriosis in humans and resistance in *C. jejuni* and *C. coli* is primarily mediated by a plasmid-encoded *tet*(O) gene (Kozak et al., 2009). In addition a

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http://dx.doi.org/10.1016/j.ijfoodmicro.2017.04.019
Received 22 July 2016; Received in revised form 21 April 2017; Accepted 28 April 2017
Available online 29 April 2017
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kanamycin resistance marker may also be present on the same plasmid (Iovine, 2013). Therefore it is important to monitor trends of antimicrobial resistance for commonly used antimicrobials.

An understanding of foodborne pathogen sources of contamination and dissemination in the food chain is key to develop effective mitigation strategies for *Campylobacter* reduction in livestock as well as prudent antimicrobial use in order to decrease the likelihood for antimicrobial resistance. In order to investigate the epidemiology of *Campylobacter* spp., molecular subtyping methods with enhanced discriminatory power are required. In recent years the Comparative Genomic Fingerprinting (CGF) technique has been used to subtype *Campylobacter jejuni* and *C. coli* (Taboada et al., 2012). This technique represents a high resolution subtyping approach which assesses genetic variability in the accessory genome. The objectives of this study were i) to determine *Campylobacter* prevalence in retail meat samples purchased in Alberta, Canada, ii) to determine the antimicrobial resistance profiles of recovered *Campylobacter* strains, and iii) to assess the genetic relatedness of retail meat *Campylobacter* strains with previously recovered clinical, animal and environmental isolates by using CGF and comparing the profiles to the Canadian CGF database in order to gain a better understanding of *Campylobacter* epidemiology.

2. Materials and methods

2.1. Sampling

Retail meat samples were obtained according to protocols of the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS, 2014). Retail meat samples consisted of ground beef, chicken, turkey, and pork. For ground beef, a systematic collection of extra-lean, lean, medium, and regular ground beef was performed. It was intended to collect chicken leg or thigh samples, however, when these parts were not available breasts or wings were collected. For turkey, leg samples were preferred, but if they were not available, samples of wings and/or ground turkey were obtained. For pork, samples of chops were obtained.

2.2. Sample preparation, processing and microbiological procedures

A total of 606 samples consisting of chicken (n = 204), turkey (n = 110), pork (147) and ground beef (n = 145) were purchased from retail stores in 2013. Upon arrival at the laboratory, samples were processed following standard protocols (CIPARS, 2014) described in the Compendium of Analytical Methods, Methods of Microbiological Analysis of Food, Health Protection Branch, Government of Canada for the isolation and identification of *Campylobacter* spp. This procedure has been validated and is routinely used by CIPARS for *Campylobacter* isolation. Briefly, one chicken leg, one turkey leg/wing, one pork chop or 25 g of ground meat (beef) were transferred to a stomacher bag containing 225 mL buffered peptone water (BPW, Oxoid Ltd., Basingstoke, UK) and mixed for 15 min. 50 mL of the sample/BPW mixture was transferred to 50 mL of double strength Bolton broth (2xBB, Oxoid Ltd.). The 2xBB was incubated under microaerophilic conditions (5% O_2, 10% CO_2 and balance N_2) (CampyPak Plus jar and CampyPak gas generator, Becton-Dickinson Microbiology Systems [BDMS], Cockeysville, MD) at 42 ± 1 °C for 48 ± 4 h. After incubation, the 2xBB was streaked for isolated colonies onto modified cefoperazone charcoal deoxycholate agar (mCCDA, Oxoid Ltd.). The mCCDA plates were incubated under microaerophilic conditions at 42 ± 1 °C for 24 to 72 h and then examined for typical colonies. These were purified using Mueller Hinton agar (MHA Oxoid Ltd.) with 5% citrated sheep blood. Colonies were tested for catalase, oxidase and Gram stain. A maximum of three colonies exhibiting reactions typical for *Campylobacter* spp. were further characterized by determining growth at 25 °C, and for cephalothin sensitivity, hippurate and indoxyl acetate hydrolysis. Additional confirmation of *Campylobacter* spp. was done using PCR with species-specific primers (Inglis and Kalischuk, 2003; Kos et al., 2006a). Up to three confirmed *Campylobacter* isolates per positive sample were stored in brain heart infusion broth supplemented with 10% blood and with 25% glycerol at − 80 °C for further analysis. Only one isolate per positive samples was used for further analyses.

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility of *Campylobacter* isolates was tested with a panel of 9 antimicrobials (Campy plates) using an automated microbroth dilution method (Sensititre®; Trek Diagnostic Systems Inc., Westlake, OH, USA) as described previously (CIPARS, 2014). Briefly, colonies were streaked onto Mueller Hinton agar plates with 5% sheep blood and incubated in a microaerophilic atmosphere at 42 ± 1 °C for 24 h. A 0.5 McFarland suspension of bacterial growth was prepared by transferring selected bacterial colonies into a tube containing 5 mL of Mueller Hinton Broth (MHB). Afterward, 10 μL of the MHB were transferred to 11 mL of MHB with laked horse blood. The mixture was dispensed onto CAMPY plates at 100 μL per well. The plates were sealed with perforated adhesive plastic sheets. After a 24 h incubation in microaerophilic atmosphere at 42 ± 1 °C, plates were read using the Sensititre Vizion System40. *Campylobacter jejuni* ATCC 33560 was used as quality control organism.

The results were interpreted by following the guidelines of the Clinical and Laboratory Standard Institute (CLSI, 2012). Where Clinical and Laboratory Standards Institute interpretive criteria were not available for Enterobacteriaceae, the antimicrobial, breakpoints were based on the distribution of minimal inhibitory concentrations and were harmonized with those of the National Antimicrobial Resistance Monitoring System (NARMS), United States. Antimicrobial susceptibility was performed for the following antimicrobial agents (antimicrobial abbreviations and breakpoints are shown in parentheses): ciprofloxacin (CIP; 0.015–64 μg/mL), telithromycin (TEL; 0.015–8 μg/mL), azithromycin (AZT; 0.015–64 μg/mL), clindamycin (CLI; 0.03–16 μg/mL), erythromycin (ERY; 0.03–64 μg/mL), gentamicin (GEN; 0.12–32 μg/mL), nalidixic acid (NAL; 4–64 μg/mL), florfenicol (FLO; 0.03–64 μg/mL), and tetracycline (TET; 0.06–64 μg/mL).

2.4. Genetic determinants of antimicrobial resistance

Confirmed *Campylobacter* isolates were screened for the presence of genetic determinants conferring antimicrobial resistance for tetracycline (*tet* O gene). DNA was obtained using a Qiagen DNA purification kit with the QIAcube® automated DNA isolation system (Qiagen Inc. Mississauga, ON, Canada), following manufacturer recommendations. PCR to detect the *tet*O gene was performed by using the primer set and PCR conditions as described previously (Gibreel et al., 2004).

2.5. Comparative genomic fingerprinting (CGF) analysis

CGF is a subtyping approach recently described that is based on assessing the presence/absence status of a set of 40 accessory genes distributed throughout the various plasticity regions in the *C. jejuni* genome (Taboada et al., 2012). To generate *Campylobacter* CGF profiles, the 40 CGF target genes were analyzed in a series of 8 multiplex PCR assays each containing 5 sets of primers along with other PCR components as previously described by (Taboada et al., 2012). The PCR results were converted to binary values with “0” representing the absence of a specific marker and “1” representing the presence of a marker. Clustering analysis was performed with Bionumerics software (v. 7.0; Applied Maths, Austin, TX, US). CGF profiles were compared to those found in the national reference CGF database, which comprises data on over 22,000 *C. jejuni* and *C. coli* isolates obtained from human clinical and non-human (environmental, animal, retail) sources collected from 1998 to date through several national and regional surveillance programs and a range of *ad hoc* sampling activities.

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