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Short communication

Prevalence and antimicrobial resistance of *Campylobacter* spp. isolated from retail chicken and duck meat in South Korea

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ABSTRACT

This study was conducted to determine the prevalence and antimicrobial resistance of *Campylobacter* isolates from chicken and duck meat in South Korea. A total of 149 *Campylobacter* spp. was isolated and 124 (66.7%) isolates were identified as *Campylobacter jejuni*, 24 (12.9%) isolates as *Campylobacter coli*, and one was unidentified. There were 102 isolates from retail duck meat with the isolation rate of 96.2%, which was significant higher ($p < 0.05$) than 47 isolates from 80 of chicken meat with the isolation rate of 58.8%. *Campylobacter* isolation rates ranged from 83.3% to 100.0% among traditional markets, wholesale markets and supermarkets; whereas the isolation rate from online store (50.0%) was significantly lower ($p < 0.01$) than the traditional markets, wholesale markets and supermarkets. Resistance to nalidixic acid, tetracycline and ciprofloxacin was most common both for chicken and duck *Campylobacter* isolates. All 24 *C. coli* isolates were resistant to tetracycline. *Campylobacter* isolates from duck had higher antibiotics resistant rates to ampicillin, ciprofloxacin, gentamicin, nalidixic acid and tetracycline, than chickens. The majority of the *Campylobacter* isolates were classified as multi-drug resistant, 57.1% of the *C. jejuni* isolates and 70.9% *C. coli* isolates were resistant to at least four antibiotics tested in this study. One *C. jejuni* isolate showed resistance to all eight antibiotics tested in this study. Our results show that retail chicken and duck meat has a high prevalence of *Campylobacter*, and the high prevalence of resistant and multi-drug resistant *Campylobacter* in retail chicken and duck meat is a potential campylobacteriosis risk for humans living in South Korea.

1. Introduction

Campylobacter is one of the most common causes of food-borne illness in humans and is the most common bacterium causing gastroenteritis worldwide. There were 212,064 confirmed cases of campylobacteriosis reported in Europe in 2010, and it has continued to be the most commonly reported gastrointestinal bacterial pathogen (EFSA, 2012). *Campylobacter jejuni* is the most common species isolated from humans, whereas *Campylobacter coli* is less frequent in causing human acute gastroenteritis (EFSA, 2012). Enteritis is the most common clinical syndrome caused by *Campylobacter*, but other extra-intestinal complications include bacteremia, reactive arthritis, and Guillain–Barre syndrome (Yuki, 2007). Infections caused by *Campylobacter* are well known and are generally transmitted through water, milk, and wild and domestic food animals, whereas poultry and poultry meat products are considered the main source of human infection (Sheppard et al., 2009). Chickens are generally considered the most common source, but there is little information concerning contamination of duck worldwide. A high prevalence of *Campylobacter* has been reported in domestic South Korean duck farms recently (Wei, Cha, et al., 2014; Wei, Huang, Liao, Liu, & Chiou, 2014). While there is little information concerning the contamination of *Campylobacter* in duck at the retail level worldwide. Fewer such studies have been performed in Asian countries, which have more than 80% of the duck meat consumption.

The majority of human infections are sporadic and self-limiting.

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Antimicrobials are not usually recommended for treatment except in severe cases and fluoroquinolones and macrolides are the preferred treatment options. Nonetheless, fluoroquinolone-resistant *Campylobacter* spp. has been reported in human infections since the 1990s and the frequency is increasing rapidly (Nachamkin, Ung, & Li, 2002). Although use of antibiotics as growth promoters has been banned in food animals in several countries including South Korea, resistant bacteria are still detected in raw meat (Ku et al., 2011). Antibiotics are used in veterinary medicine to treat sick animals or for prophylactic purposes. These antibiotics are often identical to those used in human medicine and are often of high clinical importance.

The objective of this study was to elucidate the prevalence of foodborne pathogen-*Campylobacter* in retail duck and chicken meat, and to analyze the level of antimicrobial resistance in these bacterial isolates.

2. Materials and methods

2.1. Sample collection

A total of 186 retail poultry meat samples (106 duck meat and 80 chicken samples) were collected from January to March 2013 in Jeonlado areas in South Korea (Table 1). Chicken meat included the whole body (48) and the breast (32), while duck meat included the whole body (52) and sliced samples (54). Each brand was purchased from every market, as either 1 or 2 samples if the whole body and portion both existed, to ensure complete coverage of all brands of poultry meat sold in Jeonlado. Each sample was separately retailed in a closed Styrofoam box packaged by over-wrapping with polyvinylidene film. These samples were obtained from supermarkets, traditional markets, wholesale markets, and online stores. These markets are the major sources of poultry meat for the local community. All samples purchased from supermarkets, traditional markets, and wholesale markets (where they were stored in a refrigerator) were placed immediately in a plastic bag and transported in a cool box to the laboratory. Online food shopping was that poultry meat was directly purchased from the websites of company; the meat was delivered by another logistics company who then shipped the poultry meat directly to the customer from the company' storage warehouse. All retail poultry meat purchased from online stores was kept in an airtight Styrofoam box containing ice packs and transported to the laboratory within 48 h. All meat products were kept at 0–5 °C in the warehouse for the online stores; the temperature in the airtight Styrofoam box was examined for every sample at the time of arriving in the laboratory, the sample with the temperature less than 0 °C and more than 10 °C was refused to count. Samples were stored at refrigerated temperatures (0–5 °C) until examination and *Campylobacter* isolation from all samples was commenced within 4 h of arrival in the laboratory.

2.2. Isolation and identification of Campylobacter

For isolation of *Campylobacter*, 25 g of each poultry meat samples including skin were aseptically weighted and homogenized for 2 min in a stomacher with 225 ml buffered peptone water (Difco, Sparks, MD, USA) in sterile plastic bags. Each 25 g meat sample from whole body was included the neck, wing, breast and leg including skin sample. Next, 10 ml of the homogenate was added to 10 ml of 2 × Bolton broth (Oxoid Ltd., Basingstoke, England) with Laked Horse Blood (Oxoid) supplemented with cefoperazone, vancomycin, trimethoprim, and cycloheximide (Oxoid), and incubated for 4 h at 37 °C followed by 48 h at 42 °C in a microaerophilic environment of 10% CO_2, 5% O_2, and 85% N_2. Then the enrichment was plated onto modified charcoal–cefaperazon–desoxycholate agar (mCCDA, Oxoid) containing an antibiotic supplement of cefoperazone and amphotericin (Oxoid) at 42 °C for 48 h. After incubation, the plates were examined for the typical colonies, which are generally small, gray, drop-like, and shiny. Three to five presumptive *Campylobacter* colonies from each selective agar plate were further cultured on 5% sheep blood agar plates (Komed, Seongnam, South Korea) for 24–48 h at 42 °C under microaerophilic conditions. Presumptive *Campylobacter* isolates were confirmed by PCR assay as described previously (Wei, Cha, et al., 2014; Wei, Huang, et al., 2014). After identifying each isolate, the *Campylobacter* isolates were stored in brain heart infusion broth (Oxoid) with 20% glycerol at −80 °C.

2.3. Antimicrobial susceptibility testing

The agar dilution method was used to determine susceptibility of the *Campylobacter* isolates to eight antimicrobial agents: ampicillin, azithromycin, ciprofloxacin, clindamycin, erythromycin, gentamicin, nalidixic acid, and tetracycline (all purchased from Sigma Chemical Co., St. Louis, Mo, USA). The minimum inhibitory concentrations (MICs) were determined as previously described (Wei, Cha, et al., 2014; Wei, Huang, et al., 2014). The final inoculum on the agar was approximately 1.0×10^4 CFU per spot. The breakpoints were determined according to National Antimicrobial Resistance Monitoring System (NARMS, 2010). As no ampicillin breakpoints are available for *Campylobacter*, we used the breakpoints for *Enterobacteriaceae* from the Clinical and Laboratory Standards Institute criteria (CLSI, 2011). The *C. jejuni* ATCC 33560 was used as the quality control strain. The MIC_{50} and MIC_{90} values represent the MIC value at which ≥50%/90% of the isolates in a test population are inhibited (De Melo, Figueiredo, & Ferreira-Carvalho, 2003). The multiple antibiotics resistance (MAR) index of each strain was calculated and interpreted according to the formula: a/b, a is the number of antibiotics to which a particular isolate was resistant and b is the total number of antibiotics tested (Paul, Bezbaruah, Roy, & Ghosh, 1997).

Table 1
Prevalence and distribution of *Campylobacter* species isolated from retail chicken and duck meat.

Poultry meat		No. of samples	Positive[a]	Distribution of *Campylobacter* isolates[a]		
Source	Sample type			*C. jejuni*	*C. coli*	*Campylobacter*. spp
Chicken	Whole body	48	31 (64.6)	27 (56.3)	4 (8.3)	0
	Breast	32	16 (50.0)	15 (46.9)	1 (3.1)	0
	Subtotal	80	47 (58.8)	42 (52.5)	5 (6.3)	0
Duck	Whole body	52	52 (100.0)	39 (75.0)	13 (25.0)	0
	Slice	54	50 (92.6)	43 (79.6)	6 (11.1)	1 (1.9)
	Subtotal	106	102 (96.2)	82 (77.4)	19 (17.9)	1 (0.9)
Total		186	149 (80.1)	124 (66.7)	24 (12.9)	1 (0.5)

[a] Numbers in parentheses indicate the percentages.