

Short Communication

Antimicrobial Resistance among *Campylobacter* Isolates Obtained from Retail Chicken Meat and Offal Products in Japan

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SUMMARY: A rapid increase in antimicrobial resistance in *Campylobacter* has been posing a serious concern for human health. In this study, we aimed to demonstrate the overall trend in antimicrobial resistance among *Campylobacter* isolates obtained from chicken meat and offal products collected from a wide geographic area throughout Japan. Resistance to Enrofloxacin was most frequently observed, with significantly higher rate of resistance among isolates obtained from offal (55.6%) than from meat (27.3%) samples ($p = 0.05$). These results highlight need for a better understanding of the characteristics of *Campylobacter* isolates obtained from chicken meat and offal products.

Bacteria belonging to the genus *Campylobacter*, particularly *Campylobacter jejuni*, are among the chief causes of enteritis and diarrhea in humans in many countries, including Japan (1). Moreover, the emergence of antimicrobial-resistant *Campylobacter* strains has become a threat to human health, causing potentially adverse outcomes (2). Consumption of poultry meat and offal products contaminated with *Campylobacter* is believed to be the chief route of infection (3,4). However, information pertaining to the trends in antimicrobial resistance among *Campylobacter* isolates obtained from retail poultry meat and offal products is available only for a limited geographic region in Japan (5). The present study therefore aimed to characterize antimicrobial resistance in *Campylobacter* isolates obtained from retail poultry meat and offal products collected from a wide geographical area throughout Japan.

Retail chicken meat and offal products were purchased from 9 or 10 large-scale supermarkets from each of the 5 Japanese cities (Tokyo, Sapporo, Nagoya, Osaka, and Fukuoka) between July and August 2012. A total of 102 domestic retail chicken meat, 20 imported chicken meat, and 54 domestic offal (50 livers, 2 gizzards, and 2 hearts) samples were collected in approximately equal numbers from these 5 cities. Twenty-five grams of product was taken from each pack, and the liver samples were trimmed to include biliary ducts. The weighed samples were vigorously homogenized in 50 ml of Preston enrichment medium (Oxoid, Basingstoke, UK), and 10 ml of the homogenate was enriched for 24 h at 42°C in a microaerobic atmosphere of 5% O₂, 10%

CO₂, and 85% N₂ (AnaeroPack System, Mitsubishi Gas Chemical Co. Inc., Tokyo, Japan). One loop of the enriched sample was used for inoculation of a modified charcoal-cefoperazone-deoxycholate agar plate (Merck, Darmstadt, Germany), followed by incubation for 48 h at 42°C under microaerobic conditions, as detailed above. The suspected colonies of *Campylobacter* spp. were subjected to morphological and biochemical analyses, and 2 presumptive *Campylobacter* colonies per sample were tested for *C. jejuni* and *C. coli* using a commercially available multiplex PCR system (*Campylobacter* [cdt gene] PCR Detection and Typing Kit; Takara Bio. Inc., Shiga, Japan) according to the manufacturer's instructions. The isolates confirmed by PCR as *Campylobacter* spp. were frozen at -80°C in LB broth containing 30% (v/v) glycerol and subjected to antimicrobial susceptibility testing following recovery by incubation on Muller-Hinton agar plate (Nissui Pharmaceutical Co., Tokyo, Japan) under microaerobic conditions.

A broth microdilution method was employed for testing the antimicrobial susceptibility of isolates; for this purpose, a frozen plate (Eiken Chemical Co. Ltd., Tokyo, Japan) containing cation-adjusted Mueller-Hinton broth supplemented with 2.5% lysed horse blood was utilized in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (6). Results were interpreted following incubation for 24 h at 42°C under microaerobic conditions. Correlation between isolates from equivalent products was minimized by testing 2 isolates per sample if they belonged to different species (e.g., *C. jejuni* and *C. coli*); otherwise 1 isolate was tested. The minimum inhibitory concentrations (MIC) of 7 antimicrobial agents were determined, as shown in Table 1. *C. jejuni* ATCC 33560 was employed as the quality control organism for susceptibility testing. Antimicrobial susceptibility of isolates was interpreted according to the breakpoints established by CLSI when available (i.e., EM); otherwise, criteria defined in other

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Table 1. Number of *Campylobacter jejuni* and *Campylobacter coli* with resistance against each antimicrobial isolated from retail chicken meat and offal products collected at shops in the 5 major large Japanese cities, July to August 2012

Antimicrobial (breakpoint)	<i>C. jejuni</i>			<i>C. coli</i>		
	Meat (<i>n</i> = 33)		Offal (<i>n</i> = 18)	Meat (<i>n</i> = 6)	Offal (<i>n</i> = 7)	
	<i>n</i> (%)	<i>n</i> (%)	<i>p</i> ²⁾	<i>n</i> (%)	<i>n</i> (%)	<i>p</i> ²⁾
ABPC (> 16 µg/ml) ¹⁾	3 (9.1)	2 (11.1)	0.82	1 (16.7)	0 (0)	0.46
DSM (> 16 µg/ml) ¹⁾	2 (6.1)	1 (5.6)	0.94	4 (66.7)	3 (42.9)	0.41
OTC (> 8 µg/ml) ¹⁾	7 (21.2)	7 (38.9)	0.18	6 (100)	4 (57.1)	0.19
CP (> 8 µg/ml) ¹⁾	0 (0)	1 (5.6)	0.17	0 (0)	0 (0)	na ³⁾
EM (> 16 µg/ml) ¹⁾	0 (0)	0 (0)	na ³⁾	2 (33.3)	0 (0)	0.19
ERFX (> 1 µg/ml) ¹⁾	9 (27.3)	10 (55.6)	0.05	1 (16.7)	2 (28.6)	1
NA (> 16 µg/ml) ¹⁾	8 (24.2)	10 (55.6)	0.05	1 (16.7)	2 (28.6)	1

¹⁾: Abbreviations and range of MIC testing as follows.

ABPC; Ampicillin (0.12–128 µg/ml), DSM; Dihydrostreptomycin (0.25–512 µg/ml), OTC; Oxytetracycline (0.12–64 µg/ml), CP; Chloramphenicol (0.12–64 µg/ml), EM; Erythromycin (0.12–128 µg/ml), ERFX; Enrofloxacin (0.12–64 µg/ml), NA; Nalidixic acid (0.12–256 µg/ml).

²⁾: *p*-value for significance of difference in proportion of resistance against each antimicrobial between isolates from retail meat and offal by chi-squared and Fisher's exact test for *C. jejuni* and *C. coli*, respectively.

³⁾: Not applicable because of the absence of resistant isolates.

Table 2. The ratio of proportion harboring resistance to each antimicrobial in *C. jejuni* resistant to ERFX compared to those in non-ERFX resistant isolates from retail meat and retail offal purchased at the 5 major large cities in Japan from July to August 2012 and fecal samples collected at broiler farms between 2004 and 2009 all over Japan

	ABPC +			DSM +			CP +			OTC +		
	%	Ratio (95%CI ¹⁾)	<i>p</i> ²⁾	%	Ratio (95%CI ¹⁾)	<i>p</i> ²⁾	%	Ratio (95%CI ¹⁾)	<i>p</i> ²⁾	%	Ratio (95%CI ¹⁾)	<i>p</i> ²⁾
Retail meat												
ERFX + (<i>n</i> = 9)	11.1	1.33 (0.14, 13.0)	0.8	11.1	2.67 (0.19, 38.3)	0.46	0	na ³⁾	na ³⁾	44.4	3.56 (0.98, 12.9)	0.05
ERFX – (<i>n</i> = 24)	8.3	ref.		4.2	ref.		0	ref.		12.5	ref.	
Retail offal												
ERFX + (<i>n</i> = 10)	10.0	0.8 (0.06, 10.9)	0.87	10.0	na ³⁾	1	10.0	na ³⁾	1	60.0	4.8 (0.72, 32.2)	0.06
ERFX – (<i>n</i> = 8)	12.5	ref.		0	ref.		0	ref.		12.5	ref.	
Farm fecal												
ERFX + (<i>n</i> = 36)	13.9	1.01 (0.39, 2.64)	0.98	0	0 (na ^{c)})	0.57	0	0 (na ^{c)})	1	63.9	1.64 (1.15, 2.33)	0.01
ERFX – (<i>n</i> = 95)	13.7	ref.		4.2	ref.		2.1	ref.		38.9	ref.	

¹⁾: Confidence interval.

²⁾: *p*-value of chi-squared test or Fisher's exact test.

³⁾: value unavailable because of the absence of resistant isolates.

See Table 1 for abbreviations.

Japanese studies were employed (7). Statistical analyses were performed using R version 2.15.2 (R Core Team, 2012).

Contamination with *Campylobacter jejuni* and *C. coli*, respectively, was found in 33 (32.4%) and 6 (5.9%) of the 102 domestic retail chicken meat samples, and in 18 (33.3%) and 7 (13.0%) of the samples 54 chicken offal samples; isolates were not obtained from imported chicken products. Table 1 shows the proportion of isolates from meat and offal samples that exhibit antimicrobial resistance. Resistance to Enrofloxacin (ERFX) was observed most frequently among *C. jejuni* isolates, with significantly higher resistance rates among isolates obtained from offal (55.6%) than from chicken meat (27.3%) products (*p* = 0.05). A potential explanation for this difference is disparity in the origin of the meat and offal samples; however, trends in antimicrobial resistance among isolates from offal samples have not been studied in depth to date, and further elucidation is warranted. The resistance rates to Oxytetracycline

(OTC) among *C. jejuni* isolates obtained from meat and offal samples were found to be 21.2% and 38.9%, respectively; significant differences were not found between these values. Such high rates of resistance observed against both ERFX and OTC are in line with a previous study on farm *Campylobacter* isolates (8). *C. jejuni* isolates resistant to Erythromycin (EM) were not recovered; however, 2 (33.3%) *C. coli* isolates from meat but none from offal samples showed resistance to EM. Given that resistance to ERFX was predominantly found, the occurrence of co-resistance to ERFX and another antimicrobial X was subsequently evaluated by comparing the proportion of isolates showing resistance to X among the ERFX-resistant and susceptible isolates. As a reference for co-resistance, information on 131 *C. jejuni* isolates obtained from broiler fecal samples was retrieved from the Japanese Veterinary Antimicrobial Resistance Monitoring data obtained during 2004 and 2009 (7) and presented herein. As shown in Table 2, significant co-resistance to ERFX along with OTC but not

with other antimicrobial agents was observed for all isolates obtained from meat, offal, and fecal samples. Concomitant resistance to fluoroquinolone and tetracycline has been reported frequently in recent studies (9,10). Further detailed studies involving techniques such as multilocus sequence typing are required for the elucidation of the mechanisms underlying the frequent occurrence of such co-resistance.

The isolation rate of *C. jejuni* in the present study (32.4%) is lower than that reported previously (60%) (5), which is likely attributable to the differences in the study period, experimental protocols, and target geographic area. Small sample sizes, especially for the offal isolates, may limit their representativeness; nonetheless, the present study has provided valuable information on the characteristic of antimicrobial resistance among *Campylobacter* isolates obtained from retail meat and offal products in Japan. Infection with fluoroquinolone-resistant *Campylobacter* is likely to cause comparatively severe or prolonged illness (11). The high rate of resistance to ERFX among isolates from both meat and offal products is likely to pose a great concern for public health; additional scrutiny is therefore a prerequisite for the characterization of *Campylobacter* isolates obtained from retail meat and offal products.

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Conflict of interest None to declare.

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