Biocontrol Science, 2015, Vol. 20, No. 3, 179－184

Original

Distribution of Antimicrobial Resistance in *Campylobacter* Strains Isolated from Poultry at a Slaughterhouse and Supermarkets in Japan

CHIAKI KOJIMA[1]*, MICHIRU KISHIMOTO[2], AND TAKAYUKI EZAKI[1]

[1]*Department of Microbiology, Gifu University School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan*
[2]*Nagoya University of Arts and Sciences, 57 Takenoyama Iwasaki-cho Nisshin-city, Aichi 470-0196, Japan*

Received 4 June, 2014/Accepted 12 February, 2015

Fifty strains of *Campylobacter jejuni/coli* were detected in 108 specimens of chicken meat and organs sampled at six supermarkets and one poultry slaughterhouse (large scale) between April and October 2013 (isolation rates: 84.8% from the slaughterhouse, 29.3% from the supermarkets). 46/50 strains were successfully recovered and subjected to the E-test to examine their susceptibility to three fluoroquinolone antibacterial agents authorized for use in poultry in Japan: enrofloxacin (ERFX), ofloxacin (OFLX), and norfloxacin (NLFX). 29 isolates (63%) were resistant to all three agents and 2 isolates (4.3%) were resistant to two agents (ERFX and OFLX). The resistance rates of strains isolated fom the supermarkets and slaughterhouse were 61.9% and 72.0%, respectively. Because the chickens processed at the slaughterhouse were raised without the use of fluoroquinolone, the results did not suggest a positive relationship between the use of these agents and the distribution of antimicrobial-resistant bacteria. Susceptibility to macrolide antibiotics (erythromycin [EM]) was also tested in 42 strains, and one strain (2.4%), *C. coli* from a retailer sample, showed resistance. Previous studies have detected high rates of fluoroquinolone-resistant strains, suggesting an expanding distribution of resistant bacteria. The detection of EM-resistant bacteria downstream in the food distribution chain (i.e., closer to consumers) is a concern for human health.

Key words : Campylobacter / Fluoroquinolone / Erythromycin / Antimicrobial resistance.

INTRODUCTION

Campylobacter jejuni/coli (*Campylobacter* hereafter) are bacteria which cause food poisoning. They are present in the intestines of many non-human species of animals, including poultry and other domestic animals (Igimi et al., 2012). Because *Campylobacter* was identified as the most frequent cause of bacterial food poisoning in Japan between 2003 and 2013, it has been the focus of recent preventive measures. Bacterial gastroenteritis is treated to alleviate or reduce symptoms (e.g., diarrhea, abdominal pain, and nausea). When antibiotics are used for severe cases, erythromycin (EM), a macrolide antibiotic, is the most frequent choice because of its broad antibacterial spectrum (Sakagami, 1998). In general, EM is the first choice in the treatment of Campylobacteriosis, as it is relatively easy to acquire fluoroquinolone antibacterial agents. In severe cases, however, fluoroquinolones are often used as an experimental treatment until obtaining the results regarding the causative bacteria (Tsunematsu, 2012). Therefore, increased distribution of fluoroquinolone-resistant *Campylobacter* may increase incidences of unsuccessful drug therapy. The spread of antibiotic or antimicrobial-resistant bacteria is a critical public health issue (Asai et al., 2007).

Campylobacter easily develops resistance capabilities

**Corresponding author. Tel: +81-58-230-6488, Fax: +81-58-230-4689, E-mail : r2801002(a)edu.gifu-u.ac.jp*

when fluoroquinolone antibacterial agents are used. The U.S. Food and Drug Administration has assessed the human health impact of fluoroquinolone resistant *Campylobacter*. In 2005, the administration canceled its authorization of the use of enrofloxacin (ERFX), a fluoroquinolone antibacterial agent, in poultry (Nelson et al., 2007).

In Japan, the Food Safety Commission reported that the risk people developing fluoroquinolone resistance through food is moderate (Kawasaki and Hiki, 2012). However, an assessment of resistance to fluoroquinolone used for chickens and the effect of eating such food on human health has not been completed as of 2013. Although that report describes that approximately 45% of *Campylobacter* derived from chicken sold in the market has resistance to fluoroquinolone (Igimi et al., 2008), the trend in the occurrence of resistant strains has not been confirmed.

This study aimed at researching the distribution of *Campylobacter* strains resistant to fluoroquinolones authorized for use in poultry farming in Japan, and to a macrolide antibiotic agent used to treat *Campylobacter* enteritis. We isolated *Campylobacter* from chicken obtained from the end of the food supply chain—supermarkets and a poultry slaughterhouse.

MATERIALS AND METHODS

Chicken

To isolate *Campylobacter* from chicken, we obtained 108 poultry samples from April to October, 2013. Thirty-three samples (18 from the meat: 8 thighs, 4 breasts, 4 pieces of tenderloin, 1 wing, and 1 piece of chicken fat; 15 from organs: 5 gizzards and 10 livers) were collected from chicken processed at a large-scale slaughterhouse (slaughtering 300,000 birds or more annually). The other 75 samples (41 from the meat: 15 thighs, 12 breasts, 1 sample of thigh/breast mixture, 7 pieces of tenderloin, 5 wings, and 1 piece of chicken neck meat; 34 from organs: 10 gizzards and 24 livers) were bought at six supermarkets.

Isolation and Identification of *Campylobacter*

The poultry sample (25g) was mixed with Food Pathogen Enrichment Broth (225ml) (AMR Inc.), and the mixture was shaken and cultured for 24 h at 37℃ (Hayashi et al., 2013). 25g of each of products (Nos. S201-S213) was diluted with 10×PBS and 1mL of the dilution was added to 5mL of Bolton broth (Oxoid) with 5% horse blood (Nippon Bio-test Laboratories Inc.). The broth was microaerobically cultured for 24 h at 42℃. Each enriched bacterial solution was inoculated with mCCDA (Nissui Pharmaceutical Co., Ltd.) and microaerobically cultured for 48 h at 42℃. Colonies likely to be *Campylobacter* were subjected to microscopy and the Gram stain examination, and the catalase and oxidase tests. Strains were identified as *C. jejuni* and *C. coli* when the identification rate was 95% or higher using the API Campy system (SYSMEX bioMérieux Co., Ltd.). The isolated and identified *Campylobacter* strains were stored at -80℃.

Antimicrobial Susceptibility Tests

Antimicrobial susceptibility tests were conducted with regard to three fluoroquinolones authorized for use in poultry farming—ERFX, norfloxacin (NFLX), and ofloxacin (OFLX) —and one macrolide antibiotic agent (EM). As a standard evaluation method has not been established for these agents using the disc method, the E-test (SYSMEX bioMérieux Co., Ltd.) was used instead. The E-test allows for the simple measurement of the minimum inhibitory concentration (MIC) (Kawasaki and Ono, 2009). The intermediate values between the bimodal peaks of the MICs were used for the breakpoints. For EM, disk diffusion tests were conducted using Sensi-Disc (SYSMEX bioMérieux Co., Ltd.) according to the method described by the Clinical and Laboratory Standards Institute (CLSI, 2010).

In each of the tests, pure culture colonies of *Campylobacter* with blood agar were suspended with sterilized 10×PBS, and achieved a turbidity equivalent to a 0.5 Mcfarland standard. A sterile cotton swab was dipped into the suspention and used to inoculate the surface of the Muller-Hinton Agar (KANTO Chemical Co., Inc) mixed with 5% horse blood (Nippon Bio-test Laboratories Inc.). E-test disks or Sensi-Disks were laid onto the surface of the inoculated agar plates. The agar plates were microaerobically cultured for 48h at 36℃. In addition, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Campylobacter jejuni* ATCC 33560 were used as quality control strains.

RESULTS

Isolation rates of *Campylobacter*

Table 1 shows the isolation rates of *Campylobacter* from chicken. *Campylobacter* was isolated from 50 of 108 poultry samples (46.3%; 28 from the slaughterhouse and 22 from the supermarkets). The isolation rate from the slaughterhouse products (84.8%) was three times higher than that from the supermarkets (29.3%). The isolation rate from organ samples was higher than from meat samples from both the slaughterhouse (86.7%) and the supermarkets (41.2%).

Rates of resistance to fluoroquinolone antibacterial agents

46 strains (41 *C. jejuni* and 5 *C. coli*) were success-

TABLE 1. Isolation Rates of *Campylobacter* from Various Sources and Chicken Parts.

Source	Chicken part (n)	Isolation rate (%)	Number of strains by species	
			C. jejuni	*C. coli*
Poultry slaughterhouse	Meat[a] (18)	83.3	14	1
	Organs[b] (15)	86.7	13	0
Supermarkets	Meat[c] (41)	19.5	7	1
	Organs[b] (34)	41.2	10	4
Total	(108)	46.2	44	6

Isolated *Campylobacter* from 108 samples of chicken meat and organs (33 from chickens processed at a large scale slaughterhouse, 75 samples from six supermarkets). *C. jejuni* and *C. coli* were identified by using the API Campy system (SYSMEX bioMérieux Co., Ltd.).

[a]Samples from the thigh, breast, tenderloin, wings, and fat. [b]Samples from the gizzard and liver. [c]Samples from the thigh, breast, tenderloin, wings, and neck.

fully recovered from the 50 isolates. The MIC distributions for the fluoroquinolones tested are shown in Figure 1. As MIC values for these agents showed bimodal peaks, the intermediate points were used as the breakpoints. Resistance patterns of the 46 strains (positive [+] or negative [-]) are shown in Table 2 with their sources. Antimicrobial resistance was identified in 31 strains (67.4%) to ERFX, 31 strains (67.4%) to OFLX, and 29 strains (63.0%) to NLFX. Among these strains, 29 (63.0%) showed resistance to all three agents, whereas two strains (see Table 2, strain Nos. 36 and 44) showed resistance to two agents (ERFX and OFLX). Table 3 shows the number and rates of isolated strains with resistance to fluoroquinolone antibacterial agents according to the sample by source. No significant difference in resistance was observed between the slaughterhouse strains (18; 72.0%) and the supermarket strains (13; 61.9%).

Rates of resistance to the macrolide antibiotic agent

42 *Campylobacter* strains (40 *C. jejuni* and 2 *C. coli*) were successfully recovered, and one strain (2.4%;

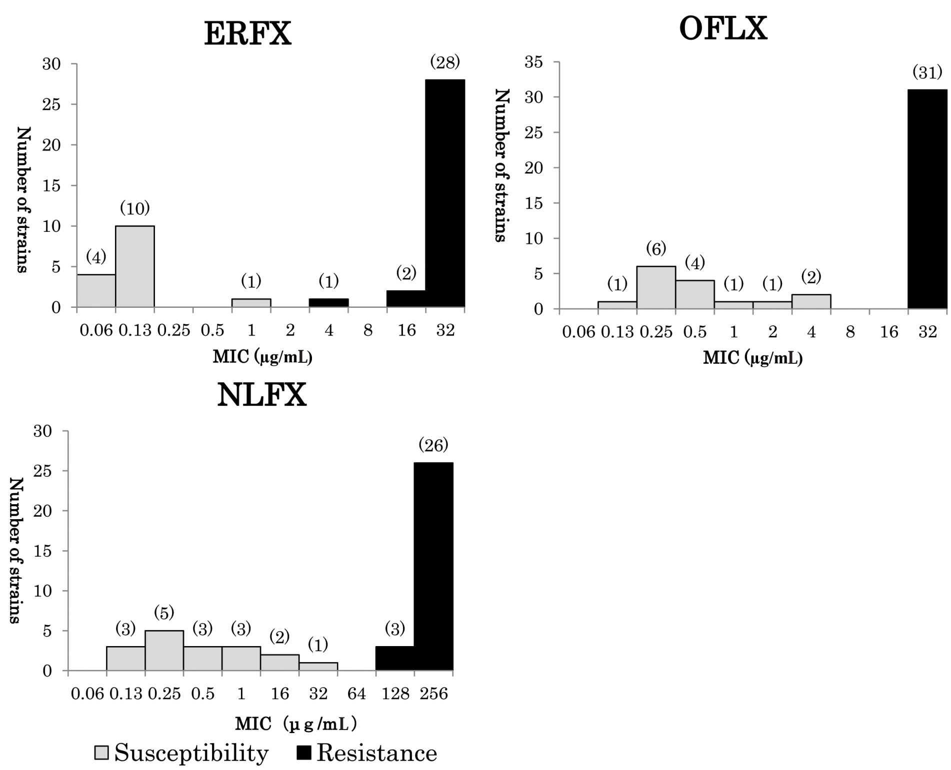

FIG. 1. The MIC distribution susceptibility to fluoroquinolone antibacterial agents of *Campylobacter*.

The MIC distribution shows the fluoroquinolone susceptibility of 46 *Campylobacter* strains. MIC values for several fluoroquinolones showed bimodal peaks, and the intermediate values were used as the breakpoints (ERFX 2μg/ml, OFLX 12μg/ml, NLFX 64μg/ml). MIC values of *Campylobacter* strains higher than the breakpoints were regarded as indicating resistance, MIC values smaller than the breakpoints as indicating susceptibility.

TABLE 2. Resistance Patterns Determined with SusceptibilityTests for Fluoroquinolone.

Campylobacter jejuni/coli	Source	Strains No.	Resistance Pattern			Sample ID	Part
			ERFX	OFLX	NLFX		
Campylobacter jejuni	Slaughter house	1	+	+	+	S15	thigh
		2	+	+	+	S16	thigh
		3	+	+	+	S25	thigh
		4	+	+	+	S27	thigh
		5	−	−	−	S3	brest
		6	−	−	−	S4	brest
		7	+	+	+	S17	brest
		8	+	+	+	S18	brest
		9	+	+	+	S19	tenderloin
		10	+	+	+	S28	tenderloin
		11	+	+	+	S29	tenderloin
		12	−	−	−	S6	wing
		13	+	+	+	S13	gizzard
		14	+	+	+	S20	gizzard
		15	+	+	+	S21	gizzard
		16	−	−	−	S33	gizzard
		17	+	+	+	S34	gizzard
		18	−	−	−	S10	liver
		19	−	−	−	S11	liver
		20	−	−	−	S12	liver
		21	+	+	+	S22	liver
		22	+	+	+	S23	liver
		23	+	+	+	S24	liver
		24	+	+	+	S30	liver
		25	+	+	+	S31	liver
	Supermarkets	26	−	−	−	R4	thigh
		27	+	+	+	R56	thigh
		28	−	−	−	R213	brest
		29	−	−	−	R60	brest
		30	+	+	+	R206	tenderloin
		31	−	−	−	R210	wing
		32	+	+	+	R7	gizzard
		33	+	+	+	R22	gizzard
		34	−	−	−	R55	gizzard
		35	−	−	−	R58	gizzard
		36	+	+	−	R62	gizzard
		37	−	−	−	R6	liver
		38	+	+	+	R24	liver
		39	−	−	−	R203	liver
		40	+	+	+	R207	liver
		41	+	+	+	R59	liver
Campylobacter coli	Supermarkets	42	+	+	+	R204	brest
		43	+	+	+	R20	gizzard
		44	+	+	−	RP3	liver
		45	+	+	+	R40	liver
		46	+	+	+	R61	liver
Total		46	31 (67.4%)	31 (67.4%)	29 (63.0%)		

Susceptibility to three fluoroquinolone antibacterial agents (ERFX, NFLX, OFLX) was tested by the E-test (SYSMEX bioMérieux Co., Ltd.) using 46 *Campylobacter* strains (41 *C. jejuni* and 5 *C. coli*).

strain No. 42 in Table 2) showed resistance to EM. This strain (*C. coli*) was isolated from a breast meat sample obtained from a supermarket and also showed resistance to the three fluoroquinolones.

TABLE 3. Resistance to Fluoroquinolone Antibacterial Agents Accoding to the Sample Source.

Source	Number (%) of strains resistant to fluoroquinolone antibacterial agents		Number (%) of resistant strains
	C. jejuni	*C. coli*	
Slaughterhouse	18 (72)	— (—)	18 (72.0)
Supermarkets	8 (50.0)	5 (100.0)	13 (61.9)
Total	26 (63.4)	5 (100.0)	31 (67.4)

Fluoroquinolone susceptibility tests with the E-test (SYSMEX bioMérieux Co., Ltd.) were conducted on 46 *Campylobacter* strains. 25 strains were isolated from slaughterhouse samples, 21 from supermarket samples. Strains showing resistance to one agent or more ware used as resistant strains.

DISCUSSION

Campylobacter grow under microaerobic conditions; they cannot grow in atmospheric conditions and die rapidly (Igimi et al., 2012). The lower isolation rates of *Campylobacter* from poultry samples in supermarkets indicate that viable cells decrease in number or die during the distribution process. This finding suggests that the fresher the meat, the higher the risk of *Campylobacter* contamination. *Campylobacter* are most prevalent in the intestines of poultry, and meat is often contaminated with intestinal contents during slaughter (Misawa, 2012). Organs are said to be at particularly high risk for contamination during their removal (Ono et al., 2002). This tendency was similarly observed in this study.

Previous studies have reported the occurrence rate of fluoroquinolone-resistant *Campylobacter* in Japan to be approximately 40% [e.g., 41.0% showing ERFX resistance in strains derived from chicken sold in the market (Food Safety Commission of Japan, 2007), 39.8% for antibacterial agent resistance in strains derived from broiler chickens (Aiba et al., 2012), and 44.1% for resistance in strains derived from chicken meat sold in the market and chicken feces (Kakimoto et al., 2007).]. In contrast, the resistance rate determined in this study was 67.4%, or approximately 1.5 times higher than previously reported rates. The administration of fluoroquinolone to livestock has been regarded as a major cause of the increase in resistant strains (Gupta et al., 2004). However, more recent studies have reported the detection of resistant strains at farms where fluoroquinolones have not been used (Asai et al., 2007; Ishihara et al., 2006). This finding indicates that the occurrence of resistant strains is not necessarily linked to the use of antibacterial agents at farms. Indeed, we detected resistant strains in poultry from a slaughterhouse that handles only chickens that have not been administered fluoroquinolone. Possible causes of this phenomenon include the influence of fluoroquinolone agents used in the past and *Campylobacter* contamination in or around the farm (Asai, 2009).

Furthermore, more scrutiny must be placed on the trend of *Campylobacter* resistance to macrolide antibiotic agents used for the treatment of *Campylobacter* enteritis (Engberg et al., 2001). Although not many cases have been reported regarding livestock-derived strains of *C. jejuni* with macrolide resistance (Haruna et al., 2001), the National Antimicrobial Resistance Monitoring System (NARMS) reported a low occurrence (0.5-1.2%) during the last 10 years (NARMS, 2012). In contrast, the prevalence of *C. coli* strains with macrolide resistance has increased after 1999 (Harada et al., 2006), albeit slightly. NARMS reported that the occurrence of macrolide resistant *C. coli* strains ranges from 4.1-9.9% with an average of 6.9% (NARMS, 2012), higher than those in *C. jejuni* strains. In this study, EM-resistant strains were isolated from chicken thigh meat samples from supermarkets, demonstrating that the distribution of resistant strains has expanded to sites accessed frequently by consumers.

The presence of these resistant bacteria raises concern about adverse effects on human health due to bacterial infection, including the possible failure of drug treatment. The occurrence of macrolide-resistant *C. jejuni* strains derived from livestock, as well as resistant *C. coli* strains, should be monitored closely, as increasing rates of macrolide antibiotic resistance, albeit at low rates, have been reported in *C. jejuni* strains isolated in clinical practice (Gibreel and Taylor, 2006). Continued studies of distribution trends and assessment of the effect on health of resistant strains of *Campylobacter* are critical.

As antibacterial agent resistance becomes more widespread, measures such as the proper use of antimicrobial agents should be implemented and even banning of their use in farms might be considered to reduce its occurrence. Furthermore, *Campylobacter* control at farms, slaughterhouses, and retail outlets are effective. Through hygienic management is needed to reduce *Campylobacter* contamination of poultry. Most slaughterhouses have a strict quality control regime. However, the processing of poultry is a large-scale operation, and therefore cross contamination occurs easily in slaughterhouses (Misawa, 2012). In this study, *Campylobacter* was isolated from the supermarket products, alert hygienic management to household and restaurants have an important part in *Campylobacter* control. In addition, *Campylobacter* food poisoning has many case of cross contamination. It is significance to promote the knowledge of preventive method of cross contamination (Igimi et al., 2012). It is critical to continue studies of distribution trends of antibacterial agent resistance and establish effective methods of *Campylobacter* control in the food processing and distribution chain.

REFERENCES

Aiba, M., Chuma, T., and Okamoto, K. (2012) Relationship of Emergence of Antimicrobial Resistance of *Campylobacter* and *Salmonella* spp. on Broiler Farms. *Journal of the Japan Veterinary Medical Association* (in Japanese), **65**, 147-152.

Asai, T. (2009) Distribution of antimicrobial resistance *Campylobacter* in broiler farms-Focus of fluoroquinolone resistance *Campylobecter. Livestock Technology* (in Japanese), **651**, 6-10.

Asai, T., Harada, K., Ishihara, K., Kojima, A., Sameshima, T., Tamura, Y., and Takahashi, T. (2007) Association of antimicrobial resistance in *Campylobacter* isolated from food-producing animals with antimicrobial use on farms. *Jpn. J. Infect. Dis*., **60**, 290-294.

Clinical and Laboratory Standards Institute. (2010) Methods for sntimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; Approved Guideline, CLSI document M45-A2.

Engberg, J., Aarestrup, F. M., Tayler, D. E., Gerner-Smidt, P., and Nachamkin, I. (2001) Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli* : Resistance mechanisms and trends in human isolates. *Emerg. Infect. Dis*., **7**, 24-34.

Food Safety Commission of Japan. (2007) Report on the Japanese food-producing animals and marine products antimicrobial resistance monitoring system. Available at: http://www.fsc.go.jp/senmon/anzenchousa/chousa20keikaku_7.pdf.

Gibreel, A., and Taylor, D. E. (2006) Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*. *J. Antimicrob. Chemother*., **58**, 243-255.

Gupta, A., Nelson, M. J., Barrett J. T., Tauxe, V. R., Rossiter, P. S., Friedman, R. C., Joyce, W. K., Smith, E. K., Jones, T. F., Hawkins, A. M., Shiferaw, B., Beebe, L. J., Vugia, J. D., Her, R. T., Benson, A. J., Root, P. T., and Angulo, J. F. (2004) Antimicrobial resistance among *Campylobacter* strains, United States, 1997-2001. *Emerg. Infect. Dis*., **10**, 1102-1109.

Harada, K., Asai, T., Kojima, A., Sameshima, T., and Takahashi, T. (2006) Characterization of macrolide-resistant *Campylobacter coli* isolates from food-producing animals on farms across Japan during 2004. *J. Bacteriol*., **68**, 1109-1111.

Haruna, M., Sasaki, Y., Murakami, M., Ikeda, A., Kusukawa, M., Tsujiyama, Y., Ito, K., Asai, T., and Yamada, Y. (2012) Prevalence and antimicrobial susceptibility of *Campylobacter* in broiler flocks in Japan. *Zoonoses. Public. Hlth*., **59**, 241-245.

Hayashi, M., Hayashi, K. S., Natori, T., Mizuno, T., Miyata, M., Yoshida, S., Zhang, J., Kawamoto, K., Ohkusu, K., Makino, S., and Ezaki, T. (2013) Use of blood-free enrichment broth in the development of rapid protocol to detect *Campylobacter* in twenty-five grams of chicken meat. *Int. J. Food. Microbiol*., **163**, 41-46.

Igimi, S., Okada, Y., Yamasaki, M., Kubo, Y., Asakura, H., and Yamamoto, S. (2008) Antimicrobial resistance of *Campylobacter*: prevalence and trends in Japan. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*, **25**, 1080-1083.

Igimi, S., Asakura, H., Okada, Y., and Momose, Y. (2012) Basic researches for the control of *Campylobacter* food poisoning. *Japanese Journal of Clinical Medicine* (in Japanese), Vol 70, No 8, 1298-1303.

Ishihara, K., Yano, S., Nishimura, M., Asai, T., Kojima, A., Takahashi, T., and TAMURA, Y. (2006) The dynamics of antimicrobial-resistant *Campylobacter jejuni* on Japanese broiler farms. *J. Vet. Med. Sci*., **68**, 515-518.

Kakimoto, S., Fukuyama, M., Furuhata, K., Oonaka, K., Yoshinami, M., Tanikawa, T., Hara, M., Harata, S., Saitoh, S., Mori, T., Muto, T., Miyai, M., and Watanabe, T., (2007) Drug aensitivity test of *Campylobacter jejuni* strains isolated from human diarrheal stools, chicken meat, and chicken feces, and gene mutation of quinolone-resistant strains. *The Journal of the Japanese Association for Infectious Diseases* (in Japanese), **81**, 363-369.

Kawakami, S., and ONO, Y. (2009) E test. *Clinical Microbiology* (in Japanese), **36**, 536-543.

Kawanishi, M., and Hiki, M. (2012) Risk management strategy of antimicrobial resistance concerning fluoroquinolone use in swine and cattle. *All about SWINE* (in Japanese), **41**, 44-47.

Misawa, N. (2012) Strategies for post-harvest control of *Campylobacter*. *Journal of the Japan Veterinary Medical Association* (in Japanese), **65**, 617-223.

National Antimicrobial Resistance Monitoring System (2012) *Retail Meat Annual Report*, 2011.Available at: http://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/UCM334834.pdf.

Nelson, J. M., Chiller, T. M., Powers, J. H., and Angulo, F. J. (2007) Fuluoroquinolone-resistant *Campylobacter* species and the withdrawal of fluoroquinolones from use in poultry: A public health success story. *Clin. Infect. Dis*., **44**, 977-980.

Ono, K., Ando, Y., Omoe, K., and Shinagawa, K. (2002) MPN and direct-plate methods used to enumerate *Campylobacter* spp. in commercial chicken livers. *Journal of the Japan Veterinary Medical Association* (in Japanese), **55**, 447-449.

Sakagami, S. (1998) Drug therapy of food poisoning. *The Journal of Practical Pharmacy* (in Japanese), **49**, 1157-1160.

Tsunematsu, S. (2012) *Campylobacter*. *Japanese Journal of Clinical Medicine* (in Japanese), **70**, 1352-1355.