Characterization of Macrolide-Resistant *Campylobacter coli* Isolates from Food-Producing Animals on Farms Across Japan during 2004

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ABSTRACT. We investigated the susceptibilities against 7 antimicrobial agents in *Campylobacter jejuni* and *C. coli* isolates from food-producing animals in 2004. In comparison with the results of past surveillance, no significant difference was observed in resistance rates against all of the antimicrobials tested in *Campylobacter* isolates. However, slight increase of erythromycin (EM) resistance was found in *C. coli* isolates from pigs. We examined the mutation of the 23S rRNA gene and their susceptibilities against azithromycin, tylosin, and lincomycin in 44 EM-resistant isolates and 28 susceptible isolates of porcine origin. All the EM-resistant isolates contained A2075G in the 23S rRNA gene and showed cross-resistance to azithromycin, tylosin, and lincomycin.

KEY WORDS: *Campylobacter coli*, erythromycin resistance, swine.

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Campylobacteriosis is among the most frequent types of bacterial food poisoning to occur in the Japanese population. Although campylobacteriosis is mainly caused by *Campylobacter jejuni*, *C. coli* is also recognized as an important food-borne pathogen [13]. Since erythromycin (EM) is the first-choice drug for the treatment of campylobacteriosis, infection with the EM-resistant *Campylobacter* spp. creates a risk of the failure of EM treatment. In this context, the emergence of EM resistance in *Campylobacter* isolates from food-producing animals poses a major problem for human medicine. In our previous study, we detected EM resistance in about half of the *C. coli* isolates examined but not in *C. jejuni*; resistance was mostly prevalent in the *C. coli* isolates from pigs on farms across Japan [7, 8].

It is known that mutations at positions 2074 and 2075 in the 23S rRNA gene are associated with EM resistance in the *Campylobacter* spp. [3–5]. However, there is little information available on the characteristics of the resistance mechanism of EM-resistant *C. coli* isolates from food-producing animals in Japan. Therefore, in the present study, we investigated the relationship between the mutation of the 23S rRNA gene in EM-resistant *C. coli* strains very recently isolated from food-producing animals in Japan and their antimicrobial susceptibilities against lincomycin and three agents belonging to the macrolide family of antibiotics.

A total of 215 *Campylobacter* isolates were obtained from 118 faecal samples of 118 apparently healthy animals (19 cattle, 42 pigs, 21 broiler chickens and 36 layer chickens) in 2004; these comprised 132 *C. jejuni* isolates (including 37, 37, and 58 isolates from cattle, broilers and layers, respectively) and 83 *C. coli* isolates (including 72 and 11 from pigs and layers, respectively). Samples were taken

from one animal per farm in 11 prefectures (Miyagi, Nagano, Ibaraki, Chiba, Toyama, Osaka, Tottori, Yamaguchi, Kochi, Kumamoto and Okinawa prefectures). Isolation of *Campylobacter* was performed using modified cefoperazone charcoal deoxycholate (mCCDA) as previously described [8], and the identification of the isolates as *C. jejuni* or *C. coli* was carried out using multiplex polymerase chain reaction (PCR) [2]. *C. jejuni* and *C. coli* were isolated in 10 and all of the 11 prefectures tested, respectively. Antimicrobial susceptibility tests were carried out by an agar dilution method as previously described [8]. The distribution of minimum inhibitory concentrations (MICs) for each antimicrobial agent is shown in Table 1. In comparison with the results of past surveillance by the Japanese Veterinary Antimicrobial Resistance Monitoring Program conducted in 1999–2001 and 2002–2003 [7, 8], no significant difference was observed in resistance rates against all of the antimicrobials tested in *Campylobacter* isolates. However, slight increase of EM resistance in *C. coli* was found in the porcine isolates: 48.3% (70/145) in 1999–2001 [7] and 48.5% (33/68) in 2002–2003 [8] to 61.1% (44/72) in 2004. Though EM resistance has been found in isolates from cattle (100% (3/3) in 1999–2001) and broiler chickens (25.0% (1/4) in 1999–2001 and 16.7% (2/12) in 2002–2003) [7, 8], no isolate of *C. coli* was obtained in the present study. In layer chickens, EM resistance was not found in 2002–2003 (0%, 0/24) [8] and in 2004, even though it was found in a small number of isolates (40.0%, 2/5) [7]. These results suggest that dynamics of EM resistance in porcine *C. coli* isolates should be noticed for a risk management of antimicrobial resistance in *Campylobacter* spp. PCR-restriction fragment length polymorphism [15] was applied for the detection of point mutations at positions 2074 (A2074C) and 2075 (A2075G) of the 23S rRNA gene in *Campylobacter* spp., which are homologous to positions 2058 and 2059 in *Escherichia coli* [15]. All of 44 EM-resistant (MIC $\geq$ 32

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Table 1. Distribution of MICs for a total of 215 *Campylobacter* isolates from food-producing animals in 2004

Agents ^{a)} (BP ^{b)})	Origins											
	Cattle (37 of <i>C. jejuni</i>)			Pigs (72 of <i>C. coli</i>)			Broilers (37 of <i>C. jejuni</i>)			Layers (58 of <i>C. jejuni</i> /11 of <i>C. coli</i>)		
	MIC ₅₀	MIC ₉₀	% Resistance	MIC ₅₀	MIC ₉₀	% Resistance	MIC ₅₀	MIC ₉₀	% Resistance	MIC ₅₀	MIC ₉₀	% Resistance
ABPC (32)	2	16	8.1	4	8	8.3	4	16	8.1	4/8	32/8	17.2/9.1
DSM (32)	0.5	16	5.4	32	> 512	54.2	0.5	1	0	0.5/1	1/1	5.2/0
GM	0.25	1	— ^{c)}	0.5	2	—	0.25	1	—	0.5/0.5	0.5/0.5	—
EM (32)	1	1	0	128	>512	61.1	1	4	0	1/1	4/8	0/0
OTC (16)	16	128	51.4	64	256	91.7	4	128	37.8	4/2	128/128	36.2/27.3
NA (32)	4	128	24.3	8	128	27.8	4	128	10.8	4/4	128/64	12.1/18.2
ERFX (2)	≤ 0.125	4	16.2	≤ 0.125	4	26.4	≤ 0.125	2	10.8	≤ 0.125/≤ 0.125	4/4	10.2/18.2

a) ABPC, ampicillin; DSM, dihydrostreptomycin; EM, erythromycin; OTC, oxytetracycline; NA, nalidixic acid; ERFX, enrofloxacin.

b) BP: Breakpoint (mg/l) was defined by Ishihara *et al.* in 2006 (8).

c) The breakpoint of gentamycin was not defined.

Table 2. Distribution of the MICs of macrolides and lincosamycin for 44 EM-resistant *C. coli* strains and 28 susceptible strains isolated from pigs in Japan

Antimicrobial agents ^{a)}	23S rRNA gene mutation	MIC ^{b)} (mg/L)													
		0.25	0.5	1	2	4	8	16	32	64	128	256	512	>512	
EM (14) ^{c)}	WT ^{d)}	1	3	4	13	3	4								
	A2075G									2	3	17	22		
AZM (15)	WT	6	12	5	5										
	A2075G											2	42		
TS (16)	WT				4	4	16		4						
	A2075G									1		9	34		
LCM	WT					5	5	11	6	1					
	A2075G										1	7	12	24	

a) EM, erythromycin; AZM, azithromycin; TS, tylosin; LCM, lincosamycin.

b) MIC, minimum inhibitory concentration.

c) Numbers in the parenthesis means lacton ring numbers of macrolide antibiotics.

d) WT, wild type.

mg/L) isolates contained the A2075G mutation but not the A2074C mutation in the 23S rDNA gene (Table 2). On the contrary, the 28 EM-susceptible isolates contained neither A2074C nor A2075G mutation. The A2075G mutation in the 23S rRNA gene had previously been found in *C. coli* isolates from pigs in New Zealand [6], Denmark [9], France [12], and U.S.A. [14]. A similar mutation has been reported in EM-resistant *C. coli* isolates from patients in Japan [10]. Our previous study suggested that *C. coli* infection in humans is contracted from pigs [8]. Although infectious enteritis in humans caused by *C. coli* occurs infrequently in Japan [11], the prevalence of EM-resistance in *C. coli* should be continuously monitored both in public health and veterinary fields.

The A2075G mutation of the 23S rRNA gene may be the predominant resistance mechanism of EM-resistant *C. coli* isolates from pigs. Cagriello *et al.* [1] have shown that EM-resistant strains with the A2075G mutation exhibited cross-resistance to 14-, 15-, and 16-member-ring macrolides. MICs of azithromycin, tylosin, and lincosamycin for 72 por-

cine *C. coli* isolates were determined as previously described [8]. As shown in Table 2, results similar to those of the previous report [1] were obtained, and the mutation was found to be involved in lincosamycin resistance. In veterinary medicine, EM, tylosin, and lincosamycin are considered important antimicrobial agents for the treatment of porcine diseases such as porcine mycoplasmosis, proliferative haemorrhagic enteropathy, and swine dysentery. However, the clinical use of macrolides and lincosamides may contribute to the increase in the EM-resistant *C. coli* strains and the maintenance of this resistance. Thus, the appropriate and prudent use of such antimicrobials is essential in the pig industry.

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