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ORIGINAL ARTICLE

Antimicrobial resistance in *Campylobacter coli* and *Campylobacter jejuni* in cynomolgus monkeys (*Macaca fascicularis*) and eradication regimens☆

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Background: Campylobacter spp. are zoonotic pathogens, however, knowledge about their presence and antimicrobial resistance in nonhuman primates is limited. Our animal facility purchased cynomolgus monkeys (*Macaca fascicularis*) from various Asian countries: China, Cambodia, Indonesia, the Philippines, and Vietnam.
Methods: Colonization by *Campylobacter* spp. was investigated in 238 of the monkeys from 2009 to 2012 and antimicrobial susceptibility testing was carried out for these isolates. Furthermore, we eradicated these pathogens from these monkeys.
Results: Campylobacter spp. were isolated from 47 monkeys from three specific countries: China, Cambodia, and Indonesia, with respective isolation rates of 15%, 36%, and 67%. Two monkeys, which were each infected with *Campylobacter jejuni* and *Campylobacter coli*, showed clinical symptoms of diarrhea and bloody feces. In total, 41 isolates of *C. coli* and 17 isolates of *C. jejuni* were detected. Antimicrobial susceptibility varied: in the monkeys from China, erythromycin (ERY)-, tetracycline (TET)-, and ciprofloxacin-resistant *C. coli*, in the monkeys from Cambodia, amoxicillin-intermediate, TET- and ciprofloxacin-resistant *C. coli* and amoxicillin- and ciprofloxacin-resistant *C. jejuni*, and in the monkeys from Indonesia,

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ciprofloxacin-resistant *C. coli* and TET- and ciprofloxacin-resistant *C. jejuni* were common (>75%). Multiresistant isolates of *C. coli* were found in monkeys from all countries and multiresistant isolates of *C. jejuni* were found in monkeys from Indonesia. The eradication rate with azithromycin was comparable to that with gentamicin (GEN) by oral administration, and was higher than those with amoxicillin-clavulanic acid (AMC) and chloramphenicol (CHL).
Conclusion: From the perspective of zoonosis, we should acknowledge multiresistant *Campylobacter* spp. isolated from the monkeys as a serious warning.

Introduction

Animals kept in captivity or bred in semi-free-range outdoor areas may become infected with enteropathogens in their enclosures.[1] Campylobacteriosis is an important zoonosis throughout the world.[2] Thermophilic *Campylobacter* spp. have been isolated from the intestinal tracts of a wide variety of animals, including poultry, swine, and captive and free-range wild animals.[3–6] *Campylobacter* spp., particularly *Campylobacter jejuni* and *Campylobacter coli*, are recognized as one of the most frequent causes of acute diarrheal disease in humans.[7]

In recent years, concern about this pathogen has increased mainly because of the frequent isolation of antimicrobial-resistant isolates in humans and animals[8] in both developed and developing countries, particularly with regard to the rapid emergence of fluoroquinolone-resistant and multidrug-resistant *Campylobacter* spp. Antimicrobial resistance in both medicine and agriculture is recognized by the World Health Organization (WHO), along with other various national authorities, as a major emerging public health concern. It represents a significant challenge of global dimensions to human and veterinary medicines with the prospect of therapeutic failure for life-saving treatments now a reality.

When cynomolgus monkeys (*Macaca fascicularis*) were introduced to our animal facility, or were checked during semi-annual health monitoring, or when abnormal gastrointestinal symptoms such as diarrhea and loose stool occurred, we checked the monkeys for the presence of *Campylobacter* spp. from 2009 to 2012 and eradicated them for the following reason: some research showed that *Campylobacter* spp. were linked to gastroenteritis in nonhuman primates.[9–11] Infected monkeys are potential reservoirs if they are asymptomatic, and they become potential transmitters of *Campylobacter* spp. to noninfected monkeys and humans who have had contact with them.[12] Moreover, infected monkeys might jeopardize the interpretation of experimental results.[13]

This study was conducted to determine the prevalence and the antimicrobial resistance of *Campylobacter* spp. and to eradicate them from cynomolgus monkeys.

Material and methods

Sample collection

Two hundred and thirty-eight cynomolgus monkeys were used in this study (Table 1). These monkeys were bred in a

Table 1 Prevalence of *Campylobacter* spp. in monkeys from different countries of origin

Country (place)	No. of monkeys		*Campylobacter* spp. positive no. of monkeys		No. of *Campylobacter* spp.		Prevalence rate (%)
	Male	Female	Male	Female	*C. coli*	*C. jejuni*	
China (Pingnan)	53	29	5	7	10	2	15
China (Hainan)	6	0	0	0	0	0	0
China (Guangxi)	5	0	0	0	0	0	0
China (unknown)	21	5	0	0	0	0	0
Cambodia (Kampong Thom)[a]	36	0	13	0	9	6	36
Indonesia (Uma Uma Island)[b]	0	33	0	22	22	9	67
Indonesia (others)	29	1	0	0	0	0	0
Philippines	1	5	0	0	0	0	0
Vietnam	11	2	0	0	0	0	0
Others	1	0	0	0	0	0	0
Total	163	75	18	29	41	17	0

[a] Prevalence rates of monkeys from Cambodia were significantly higher than those from China (Pingnan).
[b] Prevalence rates of monkeys from Indonesia were significantly higher than those from China (Pingnan) and Cambodia.

conventional and antimicrobial-free production system. They were fed a commercial diet (PS-A; Oriental Yeast Co., Ltd., Tokyo, Japan). Basically, no antibiotics were administered to them unless they were infected with bacteria and they needed the administration of antibiotic prophylaxis based on veterinary checking or symptoms. The numbers of monkeys which were introduced to our animal facility in 2009, 2010, 2011, and 2012 were 21, 27, 33, and 82, respectively, and 75 monkeys were bred prior to 2009. Most of the monkeys were purchased from Hamri Co., Ltd. (Ibaragi, Japan) and Shin Nippon Biomedical Laboratories Ltd. (Kagoshima, Japan). These breeders were imported from China (Pingnan, Hainan, Guangxi), Cambodia (Kampong Thom), Indonesia (Uma Uma Island), Indonesia (others), the Philippines, and Vietnam. The monkeys from Indonesia (Uma Uma Island) were transferred and bred in Japan (Kagoshima). In our animal facility, monkeys were housed indoors individually in standard nonhuman primate caging during quarantine and experiments. The temperature in the animal rooms was maintained at 26°C with a humidity of 45%. When the sample was collected the monkeys were aged from 2.7 years to about 20 years.

Isolation and identification of Campylobacter spp.

Fecal samples were collected when each monkey was introduced to our animal facility, when the monkey showed diarrhea or loose stool, and when they were checked during semi-annual health monitoring. The fresh feces were placed into Petri dishes and transported to the laboratory within 2 hours of collection. All samples in this study were plated directly on heart infusion agar (Difco Laboratories, Detroit, MI, USA) containing 10% defibrinated sheep blood (Nippon Bio-Test Laboratories, Inc., Tokyo, Japan) and *Campylobacter* selective supplement (Oxoid, Hampshire, England) for primary isolation. The plates were incubated in a 10% CO_2 incubator at 42°C for 36–48 hours. One to three presumptive *Campylobacter* colonies were selected for further identification by using API-Campy (BioMerieux, Marcy l'Etoile, France) kits and polymerase chain reaction (PCR) as previously described.[14] All of the isolates were stored in 10% milk at −80°C until required for further use.

Eradication regimens

After confirmation, the antibiotic disks (KB Disk Eiken: Tokyo, Japan) used in this study for drug resistance screening were as follows: amoxicillin-clavulanic acid (AMC; 20:10 μg), tetracycline (TET; 30 μg), chloramphenicol (CHL; 30 μg), gentamicin (GEN; 10 μg), erythromycin (ERY; 15 μg), and ofloxacin (5 μg). AMC (GlaxoSmithKline K.K., Tokyo, Japan), azithromycin (Pfizer Japan Inc., Tokyo, Japan), CHL (Sigma, St. Louis, MO, USA), and GEN (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were used for eradication regimens. After a drug screening test for the isolates, the monkeys were administered susceptible antimicrobials as follows: AMC at a dose of 50 mg/kg b.i.d. or 100 mg/kg b.i.d. for 5 days, azithromycin at a dose of 10 mg/kg q.d. or 20 mg/kg q.d. for 3 days, CHL at a dose of 15 mg/kg b.i.d. or 20 mg/kg b.i.d. for 5 days, or GEN at a dose of 17 mg/kg q.d., 25 mg/kg q.d., 50 mg/kg q.d., or 100 mg/kg q.d. for 5 days. Basically, azithromycin was administered to the monkeys which were infected with an ERY-susceptible isolate first, and AMC, GEN, or CHL was administered to the monkeys which were infected with an ERY-resistant isolate in the light of their susceptibility. Each antibiotic was administered in a solution of 5% methyl cellulose (1.0 mL/kg), followed by an additional 1.0 mL of the same solution to rinse the tube. After 2 weeks and 3 weeks of treatment, the feces were collected and checked for the presence of *Campylobacter* spp. Clearance was defined as the disappearance of the original isolates and eradication was defined as the disappearance of *Campylobacter* spp. both 2 weeks and 3 weeks after treatment. All experimental procedures were performed in accordance with the in-house guidelines of the Institutional Animal Care and Use Committee of Daiichi Sankyo Co., Ltd., Tokyo Japan.

Antimicrobial susceptibility testing and β-lactamase detection

The standard agar dilution method described by the Clinical and Laboratory Standards Institute (CLSI) was used to determine the minimal inhibition concentration (MIC) for *Campylobacter* spp.[15] The following is a list of antimicrobials with their abbreviations: amoxicillin (AMX), AMC, TET, CHL, GEN, amikacin (AMK), tobramycin (TOB), streptomycin (STR), neomycin (NEO), kanamycin (KAN), spectinomycin (SPT), ERY, and ciprofloxacin (CIP). All of the antimicrobials were purchased from Sigma except for clavulanic acid (Wako Pure Chemical Industries, Ltd.), TET (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), GEN (Wako Pure Chemical Industries, Ltd.), and AMK (Wako Pure Chemical Industries, Ltd.). For *C. coli* and *C. jejuni*, CLSI breakpoints were available only for TET, ERY, and CIP.[15] The CLSI breakpoints for the Enterobacteriaceae family were used for the other antimicrobials (AMX, AMC, AMK, STR, and KAN) except for TOB.[16] The National Antimicrobial Resistance Monitoring System (NARMS) breakpoints were used when CLSI breakpoints were not available (CHL and GEN).[17] The breakpoint for TOB with *Pseudomonas aeruginosa* was adopted.[18] The CLSI breakpoint for *Neisseria gonorrhea* was used for SPT.[16] Since there were no CLSI and NARMS breakpoints for NEO, we used the breakpoint for *Escherichia coli*, which is 32 μg/mL.[19] The breakpoints used for each antimicrobial are shown in Supplementary table. A *Campylobacter* isolate simultaneously resistant to three or more different classes of antimicrobials (AMX, TET, CHL, GEN, ERY, and CIP) was defined as a multidrug-resistant isolate. *C. jejuni* ATCC 33560 was used as the quality control strain. The plates were incubated at 35°C for 48 hours in a microaerobic atmosphere (AneroPak, Mitsubishi Gas Chemical Company, Inc., Tokyo, Japan) in an incubator. The MIC was defined as the lowest concentration of a drug at which no visible growth could be seen. To investigate the role of the β-lactamase in resistance to AMX, production of β-lactamase was identified by cefinase disks (Becton Dickinson and Company, Sparks, MD, USA).

Statistical analysis

Prevalence rates were analyzed by the Chi-square test for statistical analysis. The Bonferroni correction method was

used for multiple comparisons. The p values <0.05 were considered statistically significant.

Results

Campylobacter spp. prevalence

Campylobacter spp. were isolated from 47 monkeys in China (Pingnan), Cambodia (Kampong Thom), and Indonesia (Uma Uma Island; Table 1). Prevalence rates of those from China, Cambodia, and Indonesia were 15%, 36%, and 67%, respectively. Prevalence rates of those from Indonesia were significantly higher than those from China ($p < 0.0001$) and Cambodia ($p = 0.0336$), and those from Cambodia were significantly higher than those from China ($p = 0.0258$). *C. jejuni* and *C. coli* were found in two monkeys from China through semi-annual health monitoring. *C. jejuni* was found in a monkey from Cambodia which showed diarrhea and *C. coli* was found in the monkey from China which showed bloody feces. *Campylobacter* spp. were detected in the other 43 monkeys during the quarantine period. In the monkeys from China, the birth years of the positive monkeys were from 2005 to 2007, except for one monkey, with a birth year of 2003, and the range of the birth years was from 1998 to 2008. In the monkeys from Cambodia and Indonesia, the range of the birth years of the positive monkeys was from 2007 to 2008, the same as that of the birth years used for this study. Fifty-eight *Campylobacter* spp. were detected and 41 isolates of *C. coli* and 17 isolates of *C. jejuni* were identified (Table 1). In the monkeys from China, each isolate of *C. coli* and *C. jejuni* was isolated from each monkey. In the monkeys from Cambodia, both *C. coli* and *C. jejuni* were isolated from a monkey at the first isolation and two isolates of *C. coli* were recovered at the first isolation and after administration of the antimicrobial from a monkey. In the monkeys from Indonesia, two isolates of *C. coli* were isolated from a monkey at the first isolation and the monkey remained colonized with another isolate of *C. coli*. *C. coli* and *C. jejuni* were isolated at the first isolation and after administration of the antimicrobial from three monkeys. Two isolates of *C. coli* and one isolate of *C. jejuni*, and one isolate of *C. coli* and two isolates of *C. jejuni* were isolated at the first isolation and after administration of the antimicrobial from two monkeys.

Antimicrobial resistance of the Campylobacter spp. isolates

The MICs of TET, GEN, ERY, and CIP against *C. jejuni* ATCC 33560 were within the acceptable ranges. The resistance rates of antimicrobial agents for *Campylobacter* spp. are shown in Table 2. The drug resistance patterns of the *Campylobacter* spp. are shown in Table 3. The MICs and β-lactamase production of multiresistant isolates are shown in Table 4. In the monkeys from China, no AMX-, AMC-, and CHL-resistant *Campylobacter* spp. were observed. TET-resistant, CIP-resistant, and ERY-resistant *C. coli* were common (≥80%). GEN-resistant *C. coli* showed TET, AMK, TOB, STR, NEO, KAN, SPT, and CIP resistance (Table 4: H229). One of two isolates of *C. jejuni* showed susceptibility to all antimicrobials used in this study, and another isolate

Table 2 Resistance rates (%) of 13 antimicrobial agents for the *Campylobacter* spp. isolates

Campylobacter spp.	Antimicrobial agents																																						
	AMX			AMC			TET			CHL			AMK			GEN			KAN			NEO			SPT			STR			TOB			ERY			CIP		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
C. coli in China ($n = 10$)	100	0	0	100	0	0	10	0	90	90	10	0	40	30	30	80	0	20	30	0	70	30	0	70	30	0	70	60	N/A	40	30	60	20	20	0	80	0	0	100
C. coli in Cambodia ($n = 9$)	11	78	11	100	0	0	22	0	78	44	56	0	56	11	33	56	0	44	44	11	44	100	0	0	100	0	0	56	N/A	44	33	22	44	100	0	0	0	0	100
C. coli in Indonesia ($n = 22$)	91	5	5	95	5	0	41	0	59	82	14	5	64	0	36	95	0	5	59	5	36	64	0	36	64	0	36	68	N/A	32	50	45	5	68	0	32	9	0	91
C. jejuni in China ($n = 2$)	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	N/A	0	100	0	0	100	0	0	50	0	50
C. jejuni in Cambodia ($n = 6$)	17	0	83	83	17	0	83	0	13	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	N/A	0	100	0	0	100	0	0	0	0	100
C. jejuni in Indonesia ($n = 9$)	100	0	0	100	0	0	0	0	100	89	11	0	67	0	33	67	0	33	67	0	33	67	0	33	100	0	0	100	N/A	0	22	44	33	67	0	33	0	0	100

AMC = amoxicillin-clavulanic acid; AMK = amikacin; AMX = amoxicillin; CHL = chloramphenicol; CIP = ciprofloxacin; ERY = erythromycin; GEN = gentamicin; I = intermediate; KAN = kanamycin; NEO = neomycin; R = resistant; S = susceptible; SPT = spectinomycin; STR = streptomycin; TET = tetracycline; TOB = tobramycin.

Table 3 Drug resistance patterns of the *Campylobacter* spp. isolates

Campylobacter spp.	No. of resistant agents	Antimicrobial resistance patterns	No. of isolates
C. coli in China	2	TET, CIP	1
	3	TET, CIP, ERY	6
	4	TET, GEN, CIP, ERY	2
C. coli in Cambodia	2	TET, CIP	3
	2	AMX, CIP	1
	3	TET, GEN, CIP	4
C. coli in Indonesia	2	TET, CIP	5
	3	TET, CIP, ERY	7
	5	AMX, TET, CHL, GEN, CIP	1
C. jejuni in Cambodia	2	TET, CIP	1
	2	AMX, CIP	5
C. jejuni in Indonesia	2	TET, CIP	6
	4	TET, GEN, CIP, ERY	3

AMX = amoxicillin; CHL = chloramphenicol; CIP = ciprofloxacin; ERY = erythromycin; GEN = gentamicin; TET = tetracycline.

Table 4 Minimal inhibition concentrations (MICs) of 13 antimicrobial agents for multiresistant *Campylobacter* spp. and β-lactamase production

Isolate number		β-lac	AMX	AMC	TET	CHL	AMK	GEN	KAN	NEO	SPT	STR	TOB	ERY	CIP
Campylobacter coli in China	H212	−	2	2	>128	4	16	0.5	>128	>128	>128	>128	4	>128	32
	H229	−	2	2	>128	8	128	>128	>128	>128	>128	>128	>128	>128	64
C. coli in Cambodia	H468	+	16	8	>128	16	64	>128	>128	4	16	128	>128	8	128
C. coli in Indonesia	H499	−	1	1	>128	4	64	1	>128	>128	>128	2	8	>128	16
	H501	+	>128	16	>128	64	64	>128	>128	>128	>128	>128	>128	4	64
Campylobacter jejuni in Indonesia	H503	−	2	1	>128	16	64	>128	>128	>128	4	4	>128	>128	>128

AMC = amoxicillin-clavulanic acid; AMK = amikacin; AMX = amoxicillin; β-lac = β-lactamase; CHL = chloramphenicol; CIP = ciprofloxacin; ERY = erythromycin; GEN = gentamicin; KAN = kanamycin; NEO = neomycin; SPT = spectinomycin; STR = streptomycin; TET = tetracycline; TOB = tobramycin.

Table 5 Clearance and eradication rates of *Campylobacter* spp. in each regimen

Regimens	Clearance rate[a]	Eradication rate[b]
Amoxicillin-clavulanic acid 50—100 mg/kg b.i.d. for 5 d	14/15 (93)	10/15 (67)
Azithromycin 10—20 mg/kg q.d. for 3 d	26/29 (90)	24/29 (83)
Chloramphenicol 15—20 mg/kg b.i.d. for 5 d	6/8 (75)	4/8 (50)
Gentamicin 17—25 mg/kg q.d. for 5 d	2/4 (50)	1/4 (25)
Gentamicin 50—100 mg/kg q.d. for 5 d	5/5 (100)	4/5 (80)

[a] Clearance was defined as the disappearance of the original isolates and eradication was defined as the disappearance of *Campylobacter* spp. both 2 weeks and 3 weeks after treatment.
[b] Three monkeys were not eradicated because of the urgent needs for the experiments.
Data are presented as *n*/*N* (%).

showed only CIP-resistance. In the monkeys from Cambodia, no AMC-, CHL-, NEO-, SPT-, or ERY-resistant *Campylobacter* spp. were observed. TET-resistant and CIP-resistant *C. coli* were common (>75%). GEN-resistant *C. coli* showed TET, AMK, TOB, STR, KAN, and CIP resistance (Table 4: H468). One AMX-resistant isolate of *C. coli* was observed (Table 2). By contrast, AMX-resistant and CIP-resistant *C. jejuni* were common (>80%). In the monkeys from Indonesia, no AMC-resistant *Campylobacter* spp. were observed. CIP-resistant *C. coli* were common (Table 2). ERY-resistant *C. coli* showed AMK, NEO, KAN, and SPT resistance (Table 4: H499). One isolate of *C. coli* was resistant to AMX, TET, CHL, GEN, AMK, TOB, STR, NEO, KAN, SPT, and CIP (Table 4: H501). By contrast, all *C. jejuni* isolated from the monkeys from Indonesia were TET-resistant and CIP-resistant (Table 2). GEN-resistant *C. jejuni* showed TET, AMK, TOB, NEO, KAN, ERY, and CIP resistance (Table 4: H503). *Campylobacter* spp., for which the MIC for AMX was >4 μg/mL, produced β-lactamase in the monkeys from all three countries (24 isolates). Multiresistant isolates of *C. coli* were found in the monkeys from all three countries and those of *C. jejuni* were found in the monkeys from Indonesia (Table 3).

Clearance and eradication rate of antibiotics

The clearance and eradication rates in each regimen are shown in Table 5. The eradication rate of azithromycin was comparable to that of GEN by oral administration at doses of 50 mg/kg or 100 mg/kg, and higher than those of AMC and CHL. Among the eradication failures in five monkeys treated with azithromycin, ERY-resistant *C. jejuni* (MIC $>$ 128 μg/mL) appeared in two monkeys after the treatment and two *C. coli* isolates with an MIC of 8 μg/mL for ERY were still colonized. In the eradication failure in a monkey at higher doses (50 mg/kg and 100 mg/kg) of GEN treatments, an isolate with the same MIC for GEN appeared. Among the eradication failures in three monkeys at lower doses (17 mg/kg or 25 mg/kg) of GEN, a GEN-resistant isolate (MIC $>$ 128 μg/mL) appeared in a monkey after the treatment. Among the eradication failures in five monkeys treated with AMC, three isolates with higher MICs and an isolate with a lower MIC for AMC appeared, however, all isolates were susceptible to AMC. Among the eradication failures in four monkeys treated with CHL, two isolates with the same or lower MIC for CHL appeared. Most of the monkeys administered AMC and GEN showed soft feces during the treatment.

Discussion

Campylobacter spp. were isolated from three specific places: China (Pingnan), Cambodia (Kampong Thom), and Indonesia (Uma Uma Island). Interestingly, no *Campylobacter* spp. were isolated from other places even in the same country. The monkeys from China and Cambodia were bred in semi-free-range areas and the monkeys from Indonesia were bred in free-range areas. A higher percentage of the prevalence rate of the monkeys from Indonesia is attributed to the free-range environment. In semi-free-range areas, disinfected water and food washed with disinfected water were provided. Although contaminated water and food do not currently serve as a source of infection in the semi-free-range areas, there is a possibility of the origin of infection prior to disinfecting the food and water. In broiler flocks, horizontal transmission from the surrounding environment either via the farm workers or other vectors such as wild birds, vermin, and house flies, is considered to be the most likely source of contamination.[20–22] This contamination by the environment is also a potential origin of infection of the monkeys. Molecular epidemiological studies are needed to clarify this.

C. coli was the predominant species from the monkeys in all specific countries in this study. This finding is consistent with a previous report that the prevalence of *C. coli* was two times higher than that of *C. jejuni* in laboratory primates,[23] although there are few reports about the prevalence of *Campylobacter* spp. two monkeys which were infected with *C. jejuni* and *C. coli* showed clinical symptoms such as diarrhea and bloody feces, respectively; these monkeys were aged 4 years and 5 years. The other 45 monkeys did not show any clinical symptoms. These results suggested that cynomolgus monkeys at an age of $>$2.6 years old would be asymptomatic carriers of the *Campylobacter* spp. infection.

Antibiotic resistance with the fluoroquinolones and macrolide antibiotics has now emerged globally in *Campylobacter* spp.[8] and some of the resistant isolates have been reported to spread from animals to humans via the food chain.[24] Since most of the monkeys have not been exposed to antibiotics, the antibiotic resistant *Campylobacter* spp. are supposed to have been introduced from the surrounding environment. There were differences in resistant rates among the three sources, presumably reflecting differences in antibiotic use and practice. *Campylobacter* spp. are increasingly resistant to clinically important antibiotics for humans, since they are exposed to antibiotics used in both animal production and human medicine. The patterns of emerging resistance to the antimicrobial agents useful in treatment of the disease were compared in three countries. Some aminoglycosides, such as NEO and KAN, are only or often used for the animal production in some countries[25] and several aminoglycoside resistance genes have been reported.[8] Seven aminoglycosides were examined. In the monkeys from China, CIP-resistant, ERY-resistant, and TET-resistant *C. coli* were very common. In China, fluoroquinolones were used in poultry production; TET and macrolides, especially tylosin, were used as feed additives and prophylactic agents in conventional pig farms.[6] Several aminoglycoside agents, such as NEO, KAN, and SPT-resistant *C. coli* were common. This antibiotic resistance might be attributed to the sources of these animals. Only limited information is available on the prevalence and antimicrobial resistance of *Campylobacter* spp. from humans and animals in Cambodia and Indonesia.[5,26] In the monkeys from Cambodia, CIP-resistant and TET-resistant *C. coli* and AMX-resistant and CIP-resistant *C. jejuni* were common. Monitoring the retail poultry in Phnom Penh, Cambodia showed that *C. coli* were sensitive to AMX, ERY, and azithromycin and that *C. jejuni* were also sensitive to GEN.[5] In a similar fashion, *C. coli* were sensitive to ERY and *C. jejuni* were sensitive to GEN, however, the susceptibility rate of AMX was different. In the monkeys from Indonesia, CIP-resistant and TET-resistant *Campylobacter* spp. were common. Ampicillin-resistant, CIP-resistant, and TET-resistant *C. jejuni* associated with diarrheal patients have been reported in Indonesia.[26] When comparing the three countries, *C. coli* isolated from China were mostly resistant to KAN, NEO, and SPT, which were categorized in aminoglycoside groups and ERY and *Campylobacter* spp. isolated from Cambodia were mostly resistant to AMX. By contrast, the antimicrobial resistant rate ($>$1 drug) of *C. coli* isolated from Indonesia was the lowest (59%, Table 3), however, at least one isolate was resistant to all classes of antibiotics tested in this study. A surveillance program for antibiotic resistance in *Campylobacter* spp. from humans and animals in these countries, as well as the molecular mechanisms of antimicrobial resistance, is needed.

Molecular typing techniques are used to characterize the variability of an organism.[27] These techniques can also examine the relationship genotyping and antibiotic resistance in epidemiological studies. Pulsed-field gel electrophoresis fingerprinting and multilocus sequence typing would facilitate major contributions to the understanding of the epidemiology of these bacteria. Further molecular analysis for epidemiological studies is needed.

Few data were available for eradication of *Campylobacter* spp. from nonhuman primates.[28] CIP was effective in eradicating *Campylobacter* spp. of marmosets, however, treatment with either ERY or tylosin failed to eradicate *Campylobacter* spp. For clinical therapy of campylobacteriosis in humans, macrolides and fluoroquinolones are the antimicrobial agents of choice and tetracyclines have been suggested as an alternative choice.[29] Intravenous aminoglycoside therapy may also be considered in more serious cases of *Campylobacter* infections, such as bacteremia and other systemic infections.[30] It has been reported that azithromycin is clinically superior to the ERY regimen.[31] For the reasons set forth above, we first treated each monkey with azithromycin, when *Campylobacter* spp. were susceptible to ERY in a drug screening test. We applied the other drugs of choice by reference to therapy of campylobacteriosis in the clinical setting for humans. The eradication rate of azithromycin was comparable to that of GEN by oral administration at doses of 50 mg/kg or 100 mg/kg, and higher than those of AMC and CHL. Since treatment failure of azithromycin against ERY-intermediate low (MIC, 8 μg/mL) *C. coli* was observed, more doses of azithromycin or other drugs would be administered to the monkeys. When *Campylobacter* spp. were resistant to ERY and they were susceptible to AMC, GEN, or CHL, we treated the infected monkey with AMC, GEN, or CHL. In this study, since no systemic infection of monkeys was observed, oral administration of GEN was attempted. Most of the monkeys at doses of 50 mg/kg or 100 mg/kg showed soft feces, however, a higher percentage of eradication was observed. It is suggested that oral administration of GEN is one of the treatment regimens for GEN-susceptible *Campylobacter* spp. infection of monkeys. We administered relatively higher doses of AMC to the monkeys, however, the eradication rate was 80%. These doses would be optimal for eradication in the monkeys.

The Federation of European Laboratory Animal Science Association working group on nonhuman primate health reported that *C. jejuni* was classified as Group 2, which concerns agents that can cause human disease, but are unlikely to spread to the community, for which effective treatment is available.[32] They concluded the possibility that in shipments of monkeys, carriers of infectious agents transmissible to humans may be present and need to be taken into account. Since it is not easy to eradicate the antimicrobial-resistant *Campylobacter* spp. in cases of infection, we need to consider that such a risk is reduced to a sufficiently low level, such as the surveillance of the animal facility and the eradication of these pathogens.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jmii.2014.12.006.