ネコ,イヌ,ブタおよびカモメ由来thermophilic Campylobacterの抗菌剤感受性

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Antimicrobial Susceptibility of *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter laridis* from Cats, Dogs, Pigs, and Seagulls

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ABSTRACT. A total of 309 strains of thermophilic *Campylobacter* isolated from cats, dogs, pigs, and seagulls were examined for the susceptibility to 8 antimicrobial agents. The seagull strains consisting of 62 of *C. jejuni*, 31 of *C. coli*, and 34 of *C. laridis* were all susceptible to ampicillin, chloramphenicol, erythromycin, kanamycin, streptomycin, and tetracycline, showing MIC_{50}s of 0.5 to 4 μg/m*l*, MIC_{90}s of 1 to 16 μg/m*l*, and MIC ranges of ≤0.125 to 16 μg/m*l* except a few strains of *C. jejuni* and *C. laridis* which were slightly to moderately resistant to ampicillin or tetracycline. To nalidixic acid, the strains of *C. jejuni* and *C. coli* were susceptible, but those of *C. laridis* were highly resistant. To cephalothin, they were all highly resistant with MIC_{50}s of >128 μg/m*l*. In contrast, the strains consisting of 45 of *C. jejuni* from cats and dogs, 127 of *C. coli* from cats, dogs, and pigs, and 10 of *C. laridis* from dogs and pigs showed much wider MIC ranges for erythromycin, kanamycin, nalidixic acid (except *C. laridis* strains), streptomycin, and tetracycline due to the incidence of resistant strains. The incidence rates of resistance were in the range of 0 to 30% depending on the difference in the bacterial species and the origins. The highest incidence rates were observed mostly in *C. coli* strains from pigs. To ampicillin, cephalothin, and chloramphenicol, the strains represented the susceptibilities comparable to those of seagull strains.—KEY WORDS: antimicrobial susceptibility, thermophilic *Campylobacter*.

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Antimicrobial susceptibility of the thermophilic *Campylobacter* has been investigated mainly with *C. jejuni* and to a lesser extent *C. coli* from humans [6, 9, 11, 13, 18, 20, 30–32, 34]. There is concern about the development of antimicrobial resistance due to the widespread use of antimicrobial agents as therapeutic agents and as feed additives in domestic animals [1, 4, 21] from the diagnostic and epidemiological point of view. Information on the antimicrobial susceptibility of the campylobacters from animals, domestic and wild, is limited [4, 12, 13] and there are few data on that of *C. laridis* among the 3 thermophilic *Campylobacter* [24].

In this study, the antimicrobial susceptibilities of the strains of *C. jejuni*, *C. coli*, and *C. laridis* isolated from a wild animal, seagulls, and the domestic animals, cats, dogs, and pigs, were examined and compared in association with the bacterial species and the animal host.

MATERIALS AND METHODS

Bacterial strains used. A total of 309 strains of the thermophilic *Campylobacter* isolated from cats, dogs, pigs, and seagulls were used. Of these, the cat strains consisting of 26 of *C. jejuni* and 16 of *C. coli* were described previously [17]. The dog strains comprising 19 of *C. jejuni*, 8 of *C. coli*, and 2 of *C. laridis* were isolated from the fresh feces of apparently healthy dogs at Center for Animal Supply of our University, which were introduced from Animal Protection Center, Kanagawa Prefecture, Japan. The pig strains consisting of 103 of *C. coli* and 8 of *C. laridis* were isolated from the colonal contents of slaughtered pigs at an abattoir in Kanagawa Prefecture. The pig specimens were transferred to our laboratory in a vacuum bottle with ice. Isolation and identification of the dog and pig strains were carried out by the methods used formerly [17]. The seagull strains composed of 62 of *C. jejuni*, 31 of *C. coli*, and 34 of *C. laridis* were reported before [16]. Strains with negative reaction in hippurate hydrolysis and with nalidixic acid resistance, minimum inhibitory concentration (MIC) of ≥64 μg/m*l*, were identified as *C. laridis* in this study.

Staphylococcus aureus FDP 209p and *Escherichia coli* NIHJ JC2 used as control were obtained from Sankyo Co. Ltd. Tokyo, Japan.

Antimicrobial susceptibility tests. Antimicrobial susceptibility tests were performed on sensitivity test agar (Mueller-Hinton agar, modified; Nissui) by agar-dilution method. Strains to be tested were grown on heart infusion agar (Nissui) with 5% horse defibrinated blood (Nippon Bio-Test) at 37°C for 18 hr. Inoculum was prepared by suspending the growth in sterile saline. The turbidity was adjusted to a 0.5 McFarland turbidity standard. Plates with antimicrobial agents were inoculated with a multipoint replicator (Sakuma) designed to deliver 0.005 m*l* per spot to the surface of the agar. Inoculated plates were incubated in a microaerophilic atmosphere in a jar filled with O_2-CO_2-N_2 mixture (5:10:85%) at 37°C for 2 days. The control organisms, *Staphylococcus aureus* FDP 209p and *Escherichia coli* NIHJ JC2, were inoculated to each plate.

The MIC of an antimicrobial agent was defined as the lowest concentration (in micrograms per m*l* of agar) at which there was no visible growth or 10 or fewer colonies. MIC breakpoints of resistant strains were ≥16 μg/m*l* for erythromycin, streptomycin, and tetracycline and ≥32 μg/m*l* for ampicillin, cephalothin, chloramphenicol, kanamycin, and nalidixic acid in this study. Antimicrobial agents used were ampicillin (Meiji Seika), cephalothin (Sigma), chloramphenicol (Sigma), erythromycin (Sigma), kanamycin (Sigma), nalidixic acid (Sigma), streptomycin (Meiji Seika), and tetracycline (Meiji Seika).

RESULTS

For the two control strains, the MIC of each antimicrobial agent fell into the expected range of concentrations.

Table 1 shows the activity of 8 antimicrobial agents against 127 strains of *C. jejuni*, *C. coli*, and *C. laridis* from seagulls, expressed as the MIC for 50 and 90% of the strains (MIC_{50} and MIC_{90}, respectively), the MIC range, and the rate of resistance for each organism. All the 3 species of seagull *Campylobacter* strains were found to be uniformly susceptible to chloramphenicol, erythromycin, kanamycin, streptomycin, and tetracycline with almost similar MIC_{50}s of 0.5 to 4 μg/m*l*, MIC_{90}s of 1 to 8 μg/m*l*, and MIC ranges of ≤0.125 to 16 μg/m*l* except 4 strains of *C. jejuni* with an MIC of 32 μg/m*l* of tetracycline. To ampicillin, a strain of *C. jejuni* and 2 strains of *C. laridis* were slightly resistant with an MIC of 32 μg/m*l*. To cephalothin, all strains were uniformly highly resistant with the MIC_{50}s, MIC_{90}s, and MIC ranges of >128 μg/m*l*, >128 μg/m*l*, and 64 to >128 μg/m*l*, respectively. To nalidixic acid, the 3 species of seagull strains differed in the susceptibility; *C. laridis* strains were all resistant with MIC_{50} of >128 μg/m*l*, MIC_{90} of >128 μg/m*l*, and MIC range of 64 to >128 μg/m*l* while *C. jejuni* and *C. coli* strains were all susceptible with MICs of ≤16 μg/m*l*.

Table 2 shows the susceptibility to 8 antimicrobial agents of 182 strains of *C. jejuni*, *C. coli*, and *C. laridis* from cats, dogs, and pigs. They represented the MIC_{50}s, MIC_{90}s, and MIC ranges of ampicillin, chloramphenicol, and cephalothin quite similar to those of the seagull strains.

Table 1. Susceptibility of 127 strains of thermophilic *Campylobacter* isolated from seagulls to 8 antimicrobial agents

Antimicrobial agent	Organism[a)]	MIC (μg/m*l*)			
		50%	90%	Range	% Resistant strain
Ampicillin	*C. jejuni*	4	16	0.5–32	2
	C. coli	4	8	0.25–16	0
	C. laridis	4	16	1–32	6
Cephalothin	*C. jejuni*	>128	>128	64–>128	100
	C. coli	>128	>128	64–>128	100
	C. laridis	>128	>128	64–>128	100
Chloramphenicol	*C. jejuni*	4	4	0.5–16	0
	C. coli	4	4	≤0.125–8	0
	C. laridis	4	4	1–8	0
Erythromycin	*C. jejuni*	1	2	≤0.125–8	0
	C. coli	1	4	≤0.125–4	0
	C. laridis	1	4	≤0.125–4	0
Kanamycin	*C. jejuni*	2	4	0.5–16	0
	C. coli	4	8	2–16	0
	C. laridis	4	8	0.5–16	0
Nalidixic acid	*C. jejuni*	4	8	1–8	0
	C. coli	4	8	1–16	0
	C. laridis	>128	>128	64–>128	100
Streptomycin	*C. jejuni*	0.5	1	0.25–4	0
	C. coli	1	2	≤0.125–4	0
	C. laridis	1	1	0.25–2	0
Tetracycline	*C. jejuni*	0.5	1	0.25–32	6
	C. coli	0.5	1	≤0.125–2	0
	C. laridis	1	1	0.25–1	0

a) 62 of *C. jejuni*, 31 of *C. coli*, and 34 of *C. laridis* were tested.

However, in contrast to the seagull strains, they showed much wider MIC ranges for erythromycin, kanamycin, nalidixic acid (except *C. laridis* strains), streptomycin, and tetracycline due to the incidence of resistant strains. Namely, although their MIC_{50}s of 0.25 to 8 μg/m*l* were similar to those of 0.5 to 4 μg/m*l* for the seagull strains, their MIC_{90}s of 2 to 128 μg/m*l* were much higher than those of 1 to 8 μg/m*l* for the seagull strains. To nalidixic acid, the strains of *C. jejuni* and *C. coli* were susceptible except that a strain of *C. jejuni* from the cat and a strain of *C. coli* from the dog were resistant with MICs of 64 and 32 μg/m*l*, respectively, but the strains of *C. laridis* were all resistant with the MIC range of 64 to 128 μg/m*l*. The incidence rates of resistant strains against the antimicrobial agents used except cephalothin and nalidixic acid for *C. laridis* strains were in the ranges of 0 to 30% varying with the difference in antimicrobial agents, bacterial species, and animal hosts. *C. coli* strains from pigs

Table 2. Susceptibility of 182 strains of thermophilic *Campylobacter* isolated from cats, dogs, and pigs to 8 antimicrobial agents

Antimicrobial agent	Organism[a)]	Origin	MIC (μg/ml) 50%	90%	Range	% Resistant strain
Ampicillin	*C. jejuni*	Cat and dog	8	16	2–32	4
	C. coli	Cat and dog	4	16	1–32	8
	C. coli	Pig	4	8	0.25–32	2
	C. laridis	Dog and pig	4	8	0.5–16	0
Cephalothin	*C. jejuni*	Cat and dog	>128	>128	64–>128	100
	C. coli	Cat and dog	>128	>128	>128	100
	C. coli	Pig	>128	>128	>128	100
	C. laridis	Dog and pig	>128	>128	>128	100
Chloramphenicol	*C. jejuni*	Cat and dog	2	2	0.5–4	0
	C. coli	Cat and dog	2	8	0.5–8	0
	C. coli	Pig	2	4	≤0.125–64	1
	C. laridis	Dog and pig	2	4	0.25–8	0
Erythromycin	*C. jejuni*	Cat and dog	0.25	8	≤0.125–128	9
	C. coli	Cat and dog	0.5	64	≤0.125–128	17
	C. coli	Pig	0.5	128	≤0.125–>128	23
	C. laridis	Dog and pig	0.5	4	≤0.125–64	10
Kanamycin	*C. jejuni*	Cat and dog	4	8	1–128	3
	C. coli	Cat and dog	4	64	2–128	13
	C. coli	Pig	4	128	0.25–>128	21
	C. laridis	Dog and pig	4	64	0.25–128	20
Nalidixic acid	*C. jejuni*	Cat and dog	4	8	1–64	2
	C. coli	Cat and dog	8	16	2–32	4
	C. coli	Pig	4	16	0.5–16	0
	C. laridis	Dog and pig	64	128	64–128	100
Streptomycin	*C. jejuni*	Cat and dog	0.5	4	0.25–64	4
	C. coli	Cat and dog	1	64	0.5–64	13
	C. coli	Pig	2	64	≤0.125–>128	29
	C. laridis	Dog and pig	2	8	≤0.125–64	20
Tetracycline	*C. jejuni*	Cat and dog	1	32	≤0.125–64	26
	C. coli	Cat and dog	0.5	64	0.25–128	17
	C. coli	Pig	1	64	≤0.125–>128	30
	C. laridis	Dog and pig	0.5	2	0.25–32	10

a) 45 of *C. jejuni* from cats and dogs, 24 of *C. coli* from cats and dogs, and 103 of *C. coli* from pigs, and 10 of *C. laridis* from dogs and pigs were tested.

showed the highest resistance rates for erythromycin, kanamycin, streptomycin, and tetracycline among the strains used.

DISCUSSION

In this study, the antimicrobial susceptibilities of the thermophilic *Campylobacter* from the wild animal, seagulls, and the domestic animals, cats, dogs, and pigs, were compared. The strains of *C. jejuni*, *C. coli*, and *C. laridis* from cats, dogs, and pigs showed relatively wide MIC ranges particularly to erythromycin, kanamycin, streptomycin, and tetracycline with bimodal MIC distribution, possibly reflecting a development of resistance due to therapeutic and commercial drug use. This tendency of the development of resistance was conspicuous with the pig *C. coli* strains.

In contrast to the strains from cats, dogs, and pigs, most of the 3 species of seagull strains were similarly highly susceptible to the antimicrobial agents used except cephalothin and nalidixic acid for *C. laridis* strains. The origin of many genes coding for antimicrobial resistance remains speculative and also seagulls are known sometimes as a carrier of salmonellae with serotypes similar to those in the human population [7]. These may suggest that the seagull campylobacters are not exposed to any antimicrobial agents so frequently as to develop the resistance and that there is no spread of resistant campylobacters from humans and other animals at least to the particular flocks of seagulls used in this study.

However, reverse dissemination from seagulls to other animals and humans has possibly occurred [14, 15] although in the particular occasions in view of that *C. laridis*, which is found principally in seagulls [8, 10, 27], has sporadically been isolated from humans and other animals [2, 5, 24, 25, 27]. *C. laridis* was found also in the strains from dogs and pigs used in this study.

Erythromycin is regarded as a drug of choice to treat campylobacter infections in humans [3], but strains resistant to erythromycin have been observed [20, 30, 32]; the incidence rates reported for human *C. jejuni* isolates are in the range of none to 12.6% depending on the difference in geographic area. Much higher incidence rate (44%) of resistance to erythromycin was reported for swine *C. coli* [13]. Some reports [9, 23] found the erythromycin resistance only in *C. coli* not in *C. jejuni*. In this study, the erythromycin resistance was also more frequent in *C. coli* than in *C. jejuni*.

Tetracycline has been used as an alternative drug to treat campylobacter enteritis [3]. However, tetracycline resistance has been frequently observed among campylobacters from humans and other animals [4, 13, 20, 22, 29, 30, 34] and to be carried on a transferrable 30- or 48-megadalton plasmid [4, 26, 28]. High frequencies of tetracycline resistance were also observed in the strains from cats, dogs, and pigs in this study. The MICs of the resistant strains constituted the clearly separated second peak in the MIC distribution.

It has been found that *C. coli* is more resistant to many antimicrobial agents such as chloramphenicol, clindamycin, erythromycin, kanamycin, neomycin, nalidixic acid, rosaramicin, streptomycin, and tetracycline than *C. jejuni* [6, 12, 22, 32] and that plasmid DNA is observed more frequently in *C. coli* than in *C. jejuni* [4, 22]. Our results are considered to support these findings from the fact that higher frequencies of resistance to erythromycin, kanamycin, streptomycin, and tetracycline were observed in *C. coli* than in *C. jejuni* and *C. laridis*. However, no such difference was observed in seagull strains in this study.

Nalidixic acid resistance, as detected by a 30 μg disk, has been widely used as the most conspicuous characteristic in differentiating *C. laridis* from *C. jejuni* and *C. coli* [2, 25]. Recently, however, the nalidixic acid resistance has been found in occasional strains of *C. jejuni* and *C. coli* [1, 18–20, 33, 34]. In this study also, 2 strains of *C. jejuni* and *C. coli* from the cat and dog were found to be resistant to nalidixic acid.

There is little information on antimicrobial susceptibility of *C. laridis*. Simor and Wilcox [24] reported the susceptibility of a *C. laridis* strain associated with human enteritis to several antimicrobial agents. Their results are in general agreement with ours particularly of *C. laridis* strains from seagulls in this study. It was recognized from these results that the antimicrobial susceptibility of *C. laridis* is essentially identical to those of *C. coli* and *C. jejuni* except the nalidixic acid resistance which is regarded as inherent to *C. laridis*.

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要　約

ネコ，イヌ，ブタおよびカモメ由来 thermophilic *Campylobacter* の抗菌剤感受性：金内長司・足原美樹・杉山容子・今泉尚志（麻布大学獣医学部獣医公衆衛生学第二講座）——ネコ，イヌ，ブタおよびカモメ由来 thermophilic *Campylobacter* 309 株の 8 種の抗菌剤に対する感受性を調べた．カモメ由来 *C. jejuni* 62 株，*C. coli* 31 株および *C. laridis* 34 株は，ampicillin あるいは tetracycline 耐性であった 7 株を除いて，すべて ampicillin, chloramphenicol, erythromycin, kanamycin, streptomycin および tetracycline に感受性であり，cephalothin に対しては全株が高い耐性であった．また，nalidixic acid に対しては *C. jejuni* 株と *C. coli* 株はすべて感受性で，*C. laridis* 株はすべて耐性であった。ネコ，イヌおよびブタ由来の *C. jejuni* 45株，*C. coli* 127 株および *C. laridis* 10株は，erythromycin, kanamycin, nalidixic acid（*C. laridis* 株を除く），streptomycin および tetracycline に対する MIC 値が広い分布を示し，耐性株出現率は抗菌剤，菌種および動物由来別にみて 0 ～30％であり，ブタ由来 *C. coli* 株において高い傾向が認められ，ampicillin, cephalothin および chloramphenicol に対してはカモメ由来株と類似の成績を示した．