

## Antimicrobial Susceptibility Patterns of Thermophilic *Campylobacter* spp. from Humans, Pigs, Cattle, and Broilers in Denmark

FRANK MØLLER AARESTRUP,<sup>1\*</sup> EVA MØLLER NIELSEN,<sup>1</sup>  
MOGENS MADSEN,<sup>1</sup> AND JØRGEN ENGBERG<sup>2</sup>

Danish Veterinary Laboratory<sup>1</sup> and Statens Serum Institut,<sup>2</sup>  
Copenhagen, Denmark

Received 20 December 1996/Returned for modification 18 March 1997/Accepted 4 August 1997

The MICs of 16 antimicrobial agents were determined for 202 *Campylobacter jejuni* isolates, 123 *Campylobacter coli* isolates, and 6 *Campylobacter lari* isolates from humans and food animals in Denmark. The *C. jejuni* isolates originated from humans (75), broilers (95), cattle (29), and pigs (3); the *C. coli* isolates originated from humans (7), broilers (17), and pigs (99); and the *C. lari* isolates originated from broilers (5) and cattle (1). All isolates were susceptible to apramycin, neomycin, and gentamicin. Only a few *C. jejuni* isolates were resistant to one or more antimicrobial agents. Resistance to tetracycline was more common among *C. jejuni* isolates from humans (11%) than among *C. jejuni* isolates from animals (0 to 2%). More resistance to streptomycin was found among *C. jejuni* isolates from cattle (10%) than among those from humans (4%) or broilers (1%). A greater proportion of *C. coli* than of *C. jejuni* isolates were resistant to the other antimicrobial agents tested. Isolates were in most cases either coreistant to tylosin, spiramycin, and erythromycin or susceptible to all three antibiotics. More macrolide-resistant isolates were observed among *C. coli* isolates from swine (79%) than among *C. coli* isolates from broilers (18%) and humans (14%). Twenty-four percent of *C. coli* isolates from pigs were resistant to enrofloxacin, whereas 29% of *C. coli* isolates from humans and none from broilers were resistant. More resistance to streptomycin was observed among *C. coli* isolates from swine (48%) than among *C. coli* isolates from broilers (6%) or humans (0%). The six *C. lari* isolates were susceptible to all antimicrobial agents except ampicillin and nalidixic acid. This study showed that antimicrobial resistance was found only at relatively low frequencies among *C. jejuni* and *C. lari* isolates. Among *C. coli* isolates, especially from swine, there was a high level of resistance to macrolides and streptomycin. Furthermore, this study showed differences in the resistance to antimicrobial agents among *Campylobacter* isolates of different origins.

*Campylobacter* species are one of the most common causes of bacterial diarrhea in humans worldwide (13, 25). Two *Campylobacter* species are usually associated with most of the infections in man: *Campylobacter jejuni* and *Campylobacter coli* (13, 25). Patients usually recover without antimicrobial therapy, but in some patients with prolonged illness, therapy may be indicated. In these circumstances erythromycin or fluoroquinolones are often recommended (3, 8, 17, 21). *Campylobacter* infections usually occur as sporadic cases following ingestion of improperly handled or cooked food. *Campylobacteriosis* is considered a zoonotic disease, and domestic animals such as poultry, pigs, and cattle may act as reservoirs for *Campylobacter*.

*C. jejuni* isolated from clinical infections is generally susceptible to erythromycin (19, 23), whereas a higher level of resistance among isolates of *C. coli* has been reported (19, 20, 22). An increase in resistance, especially to fluoroquinolones, has been reported in several countries (7, 18, 19, 22, 29), but resistance to erythromycin and other antimicrobial agents has also been observed (19, 20, 22, 23, 29).

As *Campylobacter* may be transferred from animals to humans, the possible development of antimicrobial resistance in *Campylobacter* spp., due to the use of antimicrobial agents in food animals, is a matter of concern. It is therefore important

to know whether antimicrobial-resistant *Campylobacter* can be isolated from animals and whether these bacteria can be transferred to man.

This study was conducted to compare the frequency of isolation and the occurrence of antimicrobial resistance among different thermophilic *Campylobacter* spp. isolated in clinical infections in humans and from feces of healthy food animals in Denmark.

### MATERIALS AND METHODS

**Bacterial isolates.** A total of 82 human clinical isolates, 102 isolates from swine, 30 isolates from cattle, and 117 isolates from broilers were included in the study. Isolates from humans originated from clinical cases of diarrhea submitted to Statens Serum Institut for clinical examination. Isolates from food animals originated from fecal samples taken at slaughter from healthy animals and submitted to the Danish Veterinary Laboratory as part of a newly established surveillance scheme for antimicrobial resistance in Denmark. Only one isolate per herd or broiler flock was included in the study. All isolates were collected during 1995 and 1996. Thermophilic *Campylobacter* spp. were isolated from fecal samples of swine and cattle by selective enrichment in Preston broth (4) and incubated for 18 to 24 h at 42°C in a microaerobic atmosphere (approximately 6% O<sub>2</sub>, 7% CO<sub>2</sub>, 7% H<sub>2</sub>, 80% N<sub>2</sub>) that was created by a gas evacuation procedure (16). One loopful (10 µl) of the broth was transferred to mCCDA (Oxoid CM739 plus selective supplement SR155E). Cloacal swabs from broilers were streaked directly onto mCCDA. Agar plates were incubated at 42°C for 2 to 4 days in a microaerobic atmosphere. One isolate from each sample was identified to species level on the basis of phase-contrast microscopy (characteristic morphology and mobility), catalase, oxidase, indoxyl acetate hydrolysis, hippurate hydrolysis, and susceptibility to nalidixic acid and cephalothin (2). Only isolates identified as *C. jejuni*, *C. coli*, or *Campylobacter lari* were tested for susceptibility.

**MIC determinations.** The following antimicrobial agents were tested: ampicillin, apramycin, carbadox, chloramphenicol, colistin, enrofloxacin, erythromycin, gentamicin, nalidixic acid, neomycin, olaquinoxid, spectinomycin, spiramycin, streptomycin, tetracycline, and tylosin. The dilution ranges used for these anti-

\* Corresponding author. Mailing address: Frank Møller Aarestrup, Danish Veterinary Laboratory, 27 Bülowsvej, DK-1790 Copenhagen V, Denmark. Phone: 45 35 30 01 00. Fax: 45 35 30 01 20. E-mail: faa@svs.dk.

microbial agents were as follows: for ampicillin, apramycin, chloramphenicol, enrofloxacin, erythromycin, gentamicin, spectinomycin, and tetracycline, 0.25 to 32 µg/ml; for carbadox and olaquinox, 0.06 to 128 µg/ml; for colistin, 0.125 to 256 µg/ml; for nalidixic acid and streptomycin, 1 to 128 µg/ml; for neomycin, 0.5 to 64 µg/ml; and for tylosin, 0.5 to 128 µg/ml. MIC determinations were performed by the agar dilution method with Mueller-Hinton II agar (Becton Dickinson Microbiology Systems, Cockeysville, Md.) supplemented with 5% bovine blood. All MIC plates were inoculated with approximately 10<sup>4</sup> CFU by following the procedure of Tenover et al. (26). The plates were incubated for 48 h at 37°C in a microaerobic atmosphere (approximately 6% O<sub>2</sub>, 7% CO<sub>2</sub>, 7% H<sub>2</sub>, 80% N<sub>2</sub>). The MIC was defined as the lowest concentration producing no visible growth. The following National Committee for Clinical Laboratory Standards breakpoints for resistance (14, 15) were used: for ampicillin, chloramphenicol, and nalidixic acid, ≥32 µg/ml; for enrofloxacin, ≥2 µg/ml; for erythromycin, ≥8 µg/ml; for gentamicin, ≥16 µg/ml; and for tetracycline, ≥16 µg/ml. For the aminoglycosides apramycin, neomycin, and streptomycin, the breakpoint for gentamicin was used; for spiramycin, the breakpoint for erythromycin was used; and for spectinomycin, the breakpoint for netilmicin (≥32 µg/ml) was used. No internationally accepted breakpoints for resistance to colistin, carbadox, and olaquinox are available. For tylosin, a breakpoint of ≥64 µg/ml was used.

The following quality control strains were included on each agar plate: *Staphylococcus aureus* ATCC 25927, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27852, and *Enterococcus faecalis* ATCC 29212.

## RESULTS AND DISCUSSION

The development of antimicrobial resistance in pathogenic bacteria is a matter of increasing concern. The thermophilic *Campylobacter* species *C. jejuni*, *C. coli*, and *C. lari* can be isolated from different animal sources and may be transferred from animals to humans. Thus, the development of antimicrobial resistance in these *Campylobacter* spp., due to the use of antimicrobial agents in food animals, may have consequences for the treatment of infections in humans. Furthermore, these bacteria are frequently found in different animal sources, and they may therefore be a good choice as indicator organisms for monitoring the development of antimicrobial resistance among different animal sources.

In the present study 75 isolates from humans, 29 isolates from cattle, 95 isolates from broilers, and 3 isolates from pigs were identified as *C. jejuni*. Seven isolates from humans, 99 isolates from pigs, and 17 isolates from broilers were identified as *C. coli*, and 5 isolates from broilers and 1 isolate from cattle were identified as *C. lari*. These observations are in general agreement with those of previous studies (6, 9, 18, 19, 30) and indicate differences in the frequency of different *Campylobacter* species among the different animal sources.

MICs, MICs at which 50% of the isolates are inhibited (MIC<sub>50</sub>s), MIC<sub>90</sub>s, and percent resistant isolates are shown in Tables 1 and 2. No internationally accepted criteria for susceptibility testing or for breakpoints for susceptible versus resistant isolates are available for *Campylobacter* spp. For therapeutic agents, the breakpoints established for aerobic bacteria were used (14, 15). Tylosin has low activity under microaerophilic conditions, so a breakpoint of 64 µg/ml was used. For colistin, carbadox, and olaquinox, no breakpoints are available.

All *C. jejuni* isolates were susceptible to the aminoglycosides apramycin, neomycin, and gentamicin (Table 1). Only a limited number of *C. jejuni* isolates tested resistant to one or more antimicrobial agents. Isolates were in most cases either simultaneously resistant to the macrolide antibiotics tylosin, spiramycin, and erythromycin or susceptible to all three macrolides. However, only 7 (7%) of 95 isolates from broilers, 1 (3%) of 29 isolates from cattle, and 1 of the 3 isolates from pigs were resistant to erythromycin. This is in general agreement with the findings of previous studies among animals (5, 6, 24, 27, 29) and among humans in other countries (1, 12, 18, 19, 22–24, 29).

Resistance to tetracycline was more common among *C. jejuni* isolates from humans (11%) than among *C. jejuni* isolates

from animals (0 to 2%). A previous Danish study found all isolates susceptible to tetracycline (1), whereas studies from other countries have reported relatively high levels of resistance to tetracycline (12, 18–20, 23, 30). This could indicate some development in resistance to tetracycline among Danish human clinical isolates during the past 10 years.

More resistance to streptomycin was observed among *C. jejuni* isolates from cattle (10%) than among those from humans (4%) or broilers (1%). One of the three isolates from pigs also tested resistant to streptomycin. Relatively high levels of resistance to streptomycin among isolates from cattle have also been reported in other studies (5, 6, 27).

Increased resistance to fluoroquinolones was first reported for *Campylobacter* from chickens (7), and Jacobs-Reitsma et al. (10) reported almost 30% fluoroquinolone resistance among *Campylobacter* isolates from broilers in the Netherlands. Among isolates from humans, resistance to fluoroquinolones among *C. jejuni* isolates has emerged as a significant problem in several countries in recent years (7, 18, 19, 20, 29). However, in Sweden the level of resistance has constantly remained low (23). In the present study 9% of human clinical isolates, 7% of isolates from cattle, 14% of isolates from broilers, and one of three isolates from pigs were resistant to the fluoroquinolone enrofloxacin. This indicates that resistance to fluoroquinolones has not at present emerged as a significant problem in Denmark.

Carbadox and olaquinox are two antimicrobial agents used for growth promotion in Denmark. No breakpoints for susceptibility versus resistance are available, and no data on the susceptibility of *Campylobacter* to these agents have previously been reported. In general, carbadox showed very good activity against *C. jejuni*, with a MIC<sub>90</sub> from ≤0.06 to 2 µg/ml. However, the MIC for two isolates from humans was 32 µg/ml. Isolates could be divided into two groups, with a breakpoint of 1 µg/ml (Table 2). Olaquinox was less active than carbadox, with a MIC<sub>90</sub> from 2 to 4 µg/ml.

MICs of ampicillin were close to the breakpoint. More resistance was observed among *C. jejuni* isolates from humans than among isolates from the other animal species.

When the occurrence of resistance among *C. jejuni* isolates from the different sources was compared, the same low levels of resistance to most antimicrobial agents were observed. However, resistance to streptomycin was more frequent among *C. jejuni* isolates from cattle and resistance to tetracycline was more frequent among isolates from humans. Thus, even though the level of resistance in general was low, this indicated some differences in the susceptibility pattern among *C. jejuni* isolates of different origin.

More resistance was observed among isolates of *C. coli* than among isolates of *C. jejuni*. As with *C. jejuni*, all *C. coli* isolates were susceptible to the aminoglycosides apramycin, neomycin, and gentamicin (Table 1). Whereas most *C. jejuni* isolates were susceptible to erythromycin and the other antibiotics of the macrolide group, 1 of 7 *C. coli* isolates from humans (14%), 3 of 17 from broilers (18%), and most isolates from pigs (79%) were resistant to erythromycin. High levels of resistance to macrolides among *C. coli* isolates have also been reported in other studies (6, 9, 11, 20, 22, 28, 30). Since tylosin is widely used as a growth promoter in Denmark, the high level of macrolide resistance among isolates from pigs could be due to this selective pressure.

As also reported by Cabrera et al. (6), only a few *C. coli* isolates were resistant to tetracycline; however, this is in contrast to observations by other authors (5, 11, 20, 29). A high proportion (48%) of *C. coli* isolates from pigs were resistant to streptomycin, which is in agreement with previous studies (5, 6,

TABLE 1. MICs for 123 *C. coli* and 202 *C. jejuni* isolates from humans and animals in Denmark

Species	Antimicrobial	Origin	No. of isolates	MIC ( $\mu\text{g/ml}$ )			% Resistant <sup>d</sup>
				Range	50%	90%	
<i>C. coli</i>	Ampicillin	Humans	7	8–16	8		0
		Pigs	99	1–>32	8	32	17
		Broilers	17	2–16	4	8	0
	Apramycin	Humans	7	0.5–2	1		0
		Pigs	99	$\leq 0.25$ –8	2	4	0
		Broilers	17	0.5–4	1	2	0
	Carbadox	Humans	7	$\leq 0.06$ –0.25	0.125		NA <sup>b</sup>
		Pigs	99	0.13–8	1	4	NA
		Broilers	17	$\leq 0.06$ –2	0.13	2	NA
	Chloramphenicol	Humans	7	4–8	4		0
		Pigs	99	$\leq 0.25$ –16	4	16	12
		Broilers	17	1–8	4	8	0
	Colistin	Humans	7	1–16	2		NA
		Pigs	99	$\leq 0.25$ –32	2	16	NA
		Broilers	17	0.25–16	2	16	NA
	Enrofloxacin	Humans	7	$\leq 0.25$ –16	$\leq 0.25$		29
		Pigs	99	$\leq 0.25$ –32	$\leq 0.25$	16	13
		Broilers	17	$\leq 0.25$ –2	$\leq 0.25$	4	0
	Erythromycin	Humans	7	$\leq 0.25$ –32	1		14
		Pigs	99	$\leq 0.25$ –>32	>32	>32	74
		Broilers	17	$\leq 0.25$ –>32	1	>32	18
	Gentamicin	Humans	7	$\leq 0.25$ –0.5	0.5		0
		Pigs	99	$\leq 0.25$ –2	0.5	1	0
		Broilers	17	$\leq 0.25$ –0.5	$\leq 0.25$	$\leq 0.25$	0
	Nalidixic acid	Humans	7	4–16	8		0
		Pigs	99	$\leq 1$ –128	16	64	17
Broilers		17	4–16	8	8	0	
Neomycin	Humans	7	0.5	0.5		0	
	Pigs	99	$\leq 0.5$ –4	1	2	0	
	Broilers	17	$\leq 0.5$ –2	$\leq 0.5$	1	0	
Olaquinox	Humans	7	0.5–4	1		NA	
	Pigs	99	$\leq 0.06$ –32	2	4	NA	
	Broilers	17	0.5–8	1	2	NA	
Spectinomycin	Humans	7	2–8	8		0	
	Pigs	99	0.5–>64	8	16	4	
	Broilers	17	1–8	4	8	0	
Spiramycin	Humans	7	1–>32	1		14	
	Pigs	99	0.5–>32	>32	>32	72	
	Broilers	17	0.5–>32	1	>32	18	
Streptomycin	Humans	7	$\leq 1$	$\leq 1$		0	
	Pigs	99	$\leq 1$ –>128	4	>128	48	
	Broilers	17	$\leq 1$ –>128	$\leq 1$	2	6	
Tetracycline	Humans	7	$\leq 0.25$ –0.5	$\leq 0.25$		0	
	Pigs	99	$\leq 0.25$ –32	0.5	1	1	
	Broilers	17	$\leq 0.25$ –0.5	$\leq 0.25$	0.5	0	
Tylosin	Humans	7	4–>128	16		14	
	Pigs	99	$\leq 0.5$ –>128	>128	>128	73	
	Broilers	17	2–>128	8	>128	18	
<i>C. jejuni</i>	Ampicillin	Humans	75	1–>32	4	32	16
		Cattle	29	$\leq 0.25$ –32	8	8	3
		Broilers	95	0.5–>32	8	8	6
		Pigs	3	4–8	8		0
	Apramycin	Humans	75	$\leq 0.25$ –8	1	4	0
		Cattle	29	$\leq 0.25$ –4	1	2	0
		Broilers	95	$\leq 0.25$ –2	1	2	0
Pigs		3	1–2	1		0	

Continued on following page

TABLE 1—Continued

Species	Antimicrobial	Origin	No. of isolates	MIC ( $\mu\text{g/ml}$ )			% Resistant <sup>a</sup>
				Range	50%	90%	
Carbadox		Humans	75	$\leq 0.06$	$\leq 0.06$	$\leq 0.06$	NA
		Cattle	29	$\leq 0.06-4$	$\leq 0.06$	1	NA
		Broilers	95	$\leq 0.06-4$	1	2	NA
		Pigs	3	0.125-2	0.125		NA
Chloramphenicol		Humans	75	1-16	2	4	1
		Cattle	29	0.5-8	2	4	0
		Broilers	95	$\leq 0.25-32$	4	8	4
		Pigs	3	4-8	4		0
Colistin		Humans	75	0.5-16	8	8	NA
		Cattle	29	2-32	8	16	NA
		Broilers	95	$\leq 0.125-32$	4	16	NA
		Pigs	3	2-4	4		NA
Enrofloxacin		Humans	75	$\leq 0.25->32$	$\leq 0.25$	1	3
		Cattle	29	$\leq 0.25-16$	1	1	3
		Broilers	95	$\leq 0.25-16$	$\leq 0.25$	2	4
		Pigs	3	0.25-2	0.25		33
Erythromycin		Humans	75	$\leq 0.25-4$	1	2	0
		Cattle	29	$\leq 0.25->32$	1	1	3
		Broilers	95	$\leq 0.25->32$	1	2	6
		Pigs	3	1-16	2		33
Gentamicin		Humans	75	$\leq 0.25-1$	$\leq 0.25$	0.5	0
		Cattle	29	$\leq 0.25-2$	$\leq 0.25$	$\leq 0.5$	0
		Broilers	95	$\leq 0.25-1$	$\leq 0.25$	0.5	0
		Pigs	3	0.25-1	0.25		0
Nalidixic acid		Humans	75	4->128	8	128	12
		Cattle	29	2->128	4	>128	14
		Broilers	95	1-64	8	16	1
		Pigs	3	8-16	16		0
Neomycin		Humans	75	$\leq 0.5-2$	$\leq 0.5$	$\leq 0.5$	0
		Cattle	29	$\leq 0.5-2$	$\leq 0.5$	1	0
		Broilers	95	$\leq 0.5-2$	$\leq 0.5$	1	0
		Pigs	3	0.5-2	0.5		0
Olaquinox		Humans	75	2-4	1	2	NA
		Cattle	29	0.25-4	1	2	NA
		Broilers	95	0.25->128	1	4	NA
		Pigs	3	2-4	2		NA
Spectinomycin		Humans	75	2-64	8	16	1
		Cattle	29	$\leq 0.25-16$	8	16	0
		Broilers	95	1-16	8	8	0
		Pigs	3	8	8		0
Spiramycin		Humans	75	0.5-8	1	2	0
		Cattle	29	0.5->32	1	2	3
		Broilers	95	0.5->32	2	4	6
		Pigs	3	2-64	4		33
Streptomycin		Humans	75	$\leq 1->128$	$\leq 1$	$\leq 1$	4
		Cattle	29	$\leq 1->128$	1	32	10
		Broilers	95	$\leq 1-32$	$\leq 1$	2	1
		Pigs	3	1-32	2		33
Tetracycline		Humans	75	$\leq 0.25->32$	$\leq 0.25$	>32	11
		Cattle	29	$\leq 0.25-1$	0.25	0.5	0
		Broilers	95	$\leq 0.25-16$	$\leq 0.25$	0.5	2
		Pigs	3	0.5-1	0.5		0
Tylosin		Humans	75	8-32	16	32	0
		Cattle	29	2->128	8	16	3
		Broilers	95	0.5->128	8	64	6
		Pigs	3	16->128	>128		33

<sup>a</sup> As defined in the text.<sup>b</sup> NA, not applicable.

TABLE 2. Distribution of MICs for 123 *C. coli* and 202 *C. jejuni* isolates of human and animal origin in Denmark

Antimicrobial	Species	No. of isolates for which MIC ( $\mu\text{g/ml}$ ) is:											
		$\leq 0.06$	0.125	0.25	0.5	1	2	4	8	16	32	64	128
Ampicillin	<i>C. coli</i>					2	1	27	44	32	16	1	
	<i>C. jejuni</i>			1	4	4	12	84	75	7	11	4	
Apramycin	<i>C. coli</i>				11	33	68	10	1				
	<i>C. jejuni</i>			7	49	93	44	3	6				
Carbadox	<i>C. coli</i>	9	31	16	10	20	27	9	1				
	<i>C. jejuni</i>	128	45	10	6	1	8	2			2		
Chloramphenicol	<i>C. coli</i>			1	8	4	15	45	38	12			
	<i>C. jejuni</i>			1	13	5	74	74	29	4	2		
Colistin	<i>C. coli</i>			6	16	13	30	21	24	11	2		
	<i>C. jejuni</i>		1	1	3	9	20	62	85	19	2		
Enrofloxacin	<i>C. coli</i>			85	6	3	3	9	2	13		1	
	<i>C. jejuni</i>			104	16	58	13	2	2	6		1	
Erythromycin	<i>C. coli</i>			4	9	10	8	10	5	19	3	55	
	<i>C. jejuni</i>			13	36	110	29	5	1	1		7	
Gentamicin	<i>C. coli</i>			49	62	10	2						
	<i>C. jejuni</i>			174	21	6	1						
Nalidixic acid	<i>C. coli</i>					1	1	8	44	42	10	9	3
	<i>C. jejuni</i>					2	3	60	98	21	4	2	3
Neomycin	<i>C. coli</i>				51	45	23	4					
	<i>C. jejuni</i>				171	23	8						
Olaquinox	<i>C. coli</i>	1		1	16	46	29	22	6	1	1		
	<i>C. jejuni</i>			7	69	73	37	12	2	1			1
Spectinomycin	<i>C. coli</i>				1	4	22	12	74	6	1	3	
	<i>C. jejuni</i>			1		7	25	37	103	28		1	
Spiramycin	<i>C. coli</i>				7	16	8	9	8	12	1	62	
	<i>C. jejuni</i>				23	85	67	14	3	2	1	7	
Streptomycin	<i>C. coli</i>					34	32	8			4	11	17
	<i>C. jejuni</i>					182	12				3		17
Tetracycline	<i>C. coli</i>			53	39	24	6				1		
	<i>C. jejuni</i>			153	31	6	2			2		8	
Tylosin	<i>C. coli</i>				1		2	12	7	15	10		76
	<i>C. jejuni</i>				1		8	27	51	69	25	11	10

11), whereas only a few isolates from broilers (6%) and none from humans were resistant.

Two of seven isolates from humans tested resistant to enrofloxacin, whereas no isolates from broilers and 24% of the *C. coli* isolates from pigs tested resistant. In general, the levels of resistance to fluoroquinolones among *C. coli* isolates were higher than those for *C. jejuni*.

As with *C. jejuni*, carbadox showed good activity against *C. coli*, with a MIC<sub>90</sub> from 0.25 to 4  $\mu\text{g/ml}$ . The MIC<sub>90</sub> of olaquinox ranged from 2 to 4  $\mu\text{g/ml}$ , and MICs of ampicillin were close to the breakpoint.

When the occurrence of antimicrobial resistance among *C. coli* isolates from the different sources was compared, differences in the resistance to macrolides, enrofloxacin, and streptomycin were apparent. However, the limited number of

*C. coli* isolates from broilers and humans makes comparison difficult.

Only six *C. lari* isolates were recovered among the isolates examined in the present study. These isolates were susceptible to all antimicrobial agents tested, except for two isolates that were resistant to ampicillin and four that were resistant to nalidixic acid. Considering the limited number of isolates, it is not possible to compare levels of resistance between *C. lari* and the other *Campylobacter* species or among isolates from different animal sources.

When the antimicrobial susceptibilities for the *Campylobacter* isolates from the different sources were compared, some differences in the pattern of antimicrobial resistance among isolates were observed. *C. jejuni* was predominant among isolates from humans, cattle, and broilers but was not recovered



from pigs. *C. coli* was predominant among isolates from pigs and was recovered from humans and broilers but not from cattle, whereas *C. lari* isolates were recovered in small numbers from broilers and cattle. For *C. jejuni*, more resistance to tetracycline was observed among isolates from humans than among isolates from the different animals and more resistance to streptomycin was observed among isolates from cattle than among isolates from humans and broilers. For *C. coli*, more resistance to streptomycin and macrolides was found among isolates from pigs than among isolates from humans and broilers. Thus, for both *C. jejuni* and *C. coli*, no obvious correlation between the resistance patterns of *Campylobacter* isolates of the same species from the different animal sources and humans could be observed. This could indicate that in Denmark there are other sources than food animals for *Campylobacter* infections in humans. Furthermore, the generally low level of antimicrobial resistance among isolates from humans suggests that at present, resistance is not to a large degree transferred to or developing in *Campylobacter* isolates causing infections in humans. However, the occurrence of antimicrobial resistance genes among bacterial isolates capable of infecting humans is a matter of great concern, and the possibility that resistant bacteria or resistance genes may be transferred from animals to humans should be studied very closely. It is therefore necessary to continuously monitor the development of antimicrobial resistance.

The differences in isolation rate of the different *Campylobacter* species among the different sources may make it difficult to compare levels of resistance between the sources. Thus, it is at present difficult to say whether the higher level of macrolide resistance among *C. coli* isolates is because of their origin (most isolates are from pigs) or is related to true differences among the species. The higher level of macrolide resistance among *C. coli* isolates from humans and broilers than among *C. jejuni* isolates from the same origins indicates that there is a true difference in the ability of these species to become macrolide resistant. The possibility of isolating *C. jejuni* from humans, broilers, pigs, and cattle, and the initially low level of resistance among isolates from these sources, makes *C. jejuni* a good candidate for an indicator organism for monitoring the development of antimicrobial resistance among different sources. However, since only a few *C. jejuni* isolates were recovered from pigs, in which *C. coli* predominated, it may be difficult to compare levels of resistance among isolates from pigs with those among isolates from broilers, humans, and cattle. Thus, in order to get an acceptable prediction of resistance developing among different animal sources, it will be necessary to include more than one bacterial species.

In conclusion, this study showed that antimicrobial resistance is found only at relatively low frequencies among *C. jejuni* and *C. lari* isolates. Among *C. coli* isolates, especially from swine, there is a high level of resistance to macrolides and streptomycin. Furthermore, this study showed that, for comparison of the levels of resistance of bacterial isolates from different sources, it is important that the bacterial isolates belong to the same species, especially if any conclusions regarding zoonotic spread of resistance are to be made.

#### ACKNOWLEDGMENTS

We are grateful to René Hendriksen, Mette Juul, Sussie Kristofersen, Lissie Kjær Jensen, Inge Marianne Hansen, Karina Kristensen, Karina Absalonsen, Brit Gleerup Hansen, Annie Brandstrup, Lis Nielsen, and Gitte Lauridsen for technical assistance.

This study was part of the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP), conducted in collaboration between the Statens Serum Institut, the National Food

Agency of Denmark, and the Danish Veterinary Laboratory and funded jointly by the Danish Ministry of Health and the Danish Ministry of Food, Agriculture, and Fisheries.

#### REFERENCES

1. **Andreasen, J. J.** 1987. *In vitro* susceptibility of *Campylobacter jejuni* and *Campylobacter coli* isolated in Denmark to fourteen antimicrobial agents. *Acta Pathol. Microbiol. Immunol. Scand. Sect. B* **95**:189-192.
2. **Barrow, G. I., and R. K. A. Feltham.** 1993. Cowan and Steel's manual for the identification of medical bacteria, 3rd ed. Cambridge University Press, Cambridge, United Kingdom.
3. **Blaser, M. J.** 1990. *Campylobacter* species, p. 1649-1658. In G. L. Mandell, R. G. Douglas, and J. E. Bennett (ed.), Principles and practice of infectious diseases. Churchill Livingstone, New York, N.Y.
4. **Bolton, F. J., D. Coates, P. M. Hinchliffe, and L. Robertson.** 1983. Comparison of selective media for isolation of *Campylobacter jejuni/coli*. *J. Clin. Pathol.* **36**:78-83.
5. **Bradbury, W. C., and L. G. Munroe.** 1985. Occurrence of plasmids and antibiotic resistance among *Campylobacter jejuni* and *Campylobacter coli* isolated from healthy and diarrheic animals. *J. Clin. Microbiol.* **22**:339-346.
6. **Cabrita, J., J. Rodrigues, F. Braganca, C. Morgado, I. Pires, and A. P. Goncalves.** 1992. Prevalence, biotypes, plasmid profile and antimicrobial resistance of *Campylobacter* isolated from wild and domestic animals from Northeast Portugal. *J. Appl. Bacteriol.* **73**:279-285.
7. **Endtz, H. P., G. J. Ruijs, B. van Klingeren, W. H. Jansen, T. van der Reyden, and R. P. Mouton.** 1991. Quinolone resistance in *Campylobacter* isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. *J. Antimicrob. Chemother.* **27**:199-208.
8. **Godman, L. J., G. M. Trenholme, R. L. Kaplan, J. Segreti, D. Hines, R. Petrak, J. A. Nelson, K. W. Mayer, W. Landau, and G. W. Parkhurst.** 1990. Empiric antimicrobial therapy of domestically acquired acute diarrhea in urban adults. *Arch. Intern. Med.* **150**:541-546.
9. **Hariharan, H., T. Wright, and J. R. Long.** 1990. Isolation and antimicrobial susceptibility of *Campylobacter coli* and *Campylobacter jejuni* from slaughter hogs. *Microbiologica* **13**:1-6.
10. **Jacobs-Reitsma, W. F., P. M. F. J. Koenraad, N. M. Bolder, and R. W. A. W. Mulder.** 1994. *In vitro* susceptibility of *Campylobacter* and *Salmonella* isolates from broilers to quinolones, ampicillin, tetracycline, and erythromycin. *Vet. Q.* **16**:206-208.
11. **Kaneuchi, C., M. Ashihara, Y. Sugiyama, and T. Imaizumi.** 1988. Antimicrobial susceptibility of *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter lariidis* from cats, dogs, pigs, and seagulls. *Jpn. J. Vet. Sci.* **50**:685-691.
12. **Lariviere, L. A., C. L. Gaudreau, and F. F. Turgeon.** 1986. Susceptibility of clinical isolates of *Campylobacter jejuni* to twenty-five antimicrobial agents. *J. Antimicrob. Chemother.* **18**:681-685.
13. **Nachamkin, I.** 1995. *Campylobacter* and *Arcobacter*, p. 483-491. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
14. **National Committee for Clinical Laboratory Standards.** 1994. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; proposed standard M31-P. National Committee for Clinical Laboratory Standards, Villanova, Pa.
15. **National Committee for Clinical Laboratory Standards.** 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard M7-A4. National Committee for Clinical Laboratory Standards, Villanova, Pa.
16. **On, S. L. W., and B. Holmes.** 1991. Reproducibility of tolerance tests that are useful in the identification of *Campylobacter*. *J. Clin. Microbiol.* **29**:1785-1788.
17. **Petrucelli, B. P., G. S. Murphy, J. L. Sanchez, S. Walz, R. DeFraités, J. Gelnett, R. L. Haberberger, P. Echeverria, and D. N. Taylor.** 1992. Treatment of traveler's diarrhea with ciprofloxacin and loperamide. *J. Infect. Dis.* **165**:557-560.
18. **Rautelin, H., O.-V. Renkonen, and T. U. Kosunen.** 1991. Emergence of fluoroquinolone resistance in *Campylobacter jejuni* and *Campylobacter coli* in subjects from Finland. *Antimicrob. Agents Chemother.* **35**:2065-2069.
19. **Reina, J., M. J. Ros, and A. Serra.** 1994. Susceptibilities to 10 antimicrobial agents of 1,220 *Campylobacter* strains isolated from 1987 to 1993 from feces of pediatric patients. *Antimicrob. Agents Chemother.* **38**:2917-2920.
20. **Sagara, H., A. Muchizuki, N. Okamura, and R. Nakaya.** 1987. Antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* with special reference to plasmid profiles of Japanese clinical isolates. *Antimicrob. Agents Chemother.* **31**:713-719.
21. **Salazar-Lindo, E., R. B. Sack, E. Chea-Woo, B. A. Kay, I. Piscoya, and R. Y. Leon-Barua.** 1986. Early treatment with erythromycin of *Campylobacter jejuni* associated dysentery in children. *J. Pediatr.* **109**:3555-3560.
22. **Sánchez, R., V. Fernández-Baca, M. D. Díaz, P. Muñoz, M. Rodríguez-Créixems, and E. Bouza.** 1994. Evolution of susceptibilities of *Campylobacter* spp. to quinolones and macrolides. *Antimicrob. Agents Chemother.* **38**:1879-1882.

23. Sjøgren, E., B. Kaijser, and M. Werner. 1992. Antimicrobial susceptibilities of *Campylobacter jejuni* and *Campylobacter coli* isolated in Sweden: a 10-year follow-up report. *Antimicrob. Agents Chemother.* **36**:2847–2849.
24. Svedhem, Å., B. Kaijser, and E. Sjøgren. 1981. Antimicrobial susceptibility of *Campylobacter jejuni* isolated from humans with diarrhoea and from healthy chickens. *J. Antimicrob. Chemother.* **7**:301–305.
25. Taylor, D. N., and M. J. Blaser. 1991. *Campylobacter* infections, p. 151–172. In A. S. Evans and P. S. Brachman (ed.), *Bacterial infections in humans*. Plenum Publishing Corp., New York, N.Y.
26. Tenover, F. C., C. N. Baker, C. L. Fennell, and C. A. Ryan. 1992. Antimicrobial resistance in *Campylobacter* species, p. 66–73. In I. Nachamkin, M. J. Blaser, and L. S. Tompkins (ed.), *Campylobacter jejuni*: current status and future trends. American Society for Microbiology, Washington, D.C.
27. Vanhoof, R., H. Goossens, H. Coignau, G. Stas, and J. P. Butzler. 1982. Susceptibility pattern of *Campylobacter jejuni* from human and animal origins to different antimicrobial agents. *Antimicrob. Agents Chemother.* **21**:990–992.
28. Varoli, O., M. Gatti, M. T. Montella, and M. La Placa, Jr. 1991. Observations made on strains of *Campylobacter* spp. isolated in 1989 in Northern Italy. *Microbiologica* **14**:31–35.
29. Velázquez, J. B., A. Jiménez, B. Chomón, and T. G. Villa. 1995. Incidence and transmission of antibiotic resistance in *Campylobacter jejuni* and *Campylobacter coli*. *J. Antimicrob. Chemother.* **35**:173–178.
30. Wang, W.-L. L., L. B. Reller, and M. J. Blaser. 1984. Comparison of antimicrobial susceptibility patterns of *Campylobacter jejuni* and *Campylobacter coli*. *Antimicrob. Agents Chemother.* **26**:351–353.