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Isolation and Characterization of Cephalothin-Susceptible *Campylobacter coli* from Slaughter Cattle

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In a recent meat survey, 10 of 13 (77%) *Campylobacter coli* isolates were susceptible to cephalothin. These organisms were isolated from nine slaughter cattle from eight meat packing establishments. All 10 isolates grew at 43°C but not at 25°C, were catalase and oxidase positive, and were susceptible to nalidixic acid (30 μg) and cephalothin (30 μg). The cultures were subsequently identified as *C. coli* serogroup 20, biotype I (Lior scheme). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed that protein and lipopolysaccharide profiles of whole cell preparations of the 10 cephalothin-susceptible strains and the reference strain for *C. coli* serogroup 20 were very similar. The plasmid profiles of these 11 strains were identical.

Several studies (6, 12) have provided evidence that thermophilic campylobacters are resistant to cephalothin. This resistance has been widely used as a key diagnostic characteristic to differentiate *Campylobacter coli* and *Campylobacter jejuni* from *Campylobacter fetus* (13, 20, 25). However, in a survey from 1983 to 1984 (unpublished data) of beef carcasses from meat packing establishments in southern Ontario, 10 of 13 *C. coli* isolates were observed to be susceptible to cephalothin on initial screening. Also, one *C. coli* reference strain originally reported to be cephalothin resistant was found to be cephalothin susceptible after continued subculture.

The purpose of this study was to characterize these cephalothin-susceptible isolates on the basis of antimicrobial susceptibility patterns and protein, lipopolysaccharide (LPS), and plasmid profiles. Selected cephalothin-resistant strains of *C. coli* were included for comparison.

MATERIALS AND METHODS

Isolation of *C. coli* and *C. jejuni* from slaughter cattle. A survey of beef carcasses to determine the prevalence of thermophilic campylobacters was conducted in 16 randomly selected meat packing plants in southern Ontario. Neck muscles and gall bladders from 85 carcasses and neck muscles only from an additional 7 carcasses were tested. An enrichment suspension was prepared by aseptically transferring a 25-g sample of neck muscle or gall bladder to a stomacher bag containing approximately 100 ml of Rosef enrichment broth (24) and then stomaching the contents for 1 min in a Colworth Stomacher 400 (10). The enrichment suspension was incubated at 42°C for 24 h in a microaerophilic gas mixture of 10% CO_2–4% O_2–86% N_2. A sample was then inoculated onto Mueller-Hinton agar (Oxoid Canada, Nepean, Ontario, Canada) containing 10% sheep blood and the following supplements: trimethoprim lactate (5 mg/liter), vancomycin (10 mg/liter), polymyxin B (2,500 IU/liter), and sodium pyruvate (0.5 g/liter). The plates were incubated at 43°C for 48 h in the microaerophilic gas mixture and then examined. The methods for identification, biotyping, and serotyping were as described by Garcia et al. (10). Briefly, cultures used in the various tests were grown on Mueller-Hinton blood agar at 43°C for 24 h in the microaerophilic gas mixture. The following tests were performed: Gram stain, motility, incubation at 25°C, catalase, oxidase, and susceptibility to nalidixic acid and cephalothin. Biotyping was performed on the basis of three metabolic tests: sodium hippurate hydrolysis, H_2S production, and DNA hydrolysis (16). Serotyping with crude and absorbed sera was by the slide agglutination method of Lior et al. (17).

Bacterial strains. The campylobacter strains used are shown in Table 1. *C. coli* C-793-1 to C-793-10 and C-152-1, C-152-2, and C-152-6 were isolated in this study. *C. coli* C-602S is the reference strain for serogroup 20 in the Lior serotyping scheme (17) and is susceptible to cephalothin. *C. coli* C-602R is a cephalothin-resistant strain. *C. coli* C-602S and C-602R were derived from the same isolate.

Antimicrobial susceptibility testing. Antimicrobial susceptibility tests were conducted essentially by the disk diffusion method described by Barry and Thornsberry (1), except that Mueller-Hinton agar (Oxoid) plus 10% sheep blood and growth for 48 h under microaerophilic conditions were used. The following Oxoid antimicrobial disks were used: nalidixic acid, 30 μg; cephalothin, 30 μg; gentamicin, 10 μg; tetracycline, 30 μg; erythromycin, 15 μg; ampicillin, 10 μg; clindamycin, 2 μg; streptomycin, 10 μg; sulfamethoxazole-trimethoprim, 25 μg; and metronidazole, 5 μg.

SDS-PAGE. Organisms were grown on plates with Mueller-Hinton agar plus 10% sheep blood for 2 to 3 days at 43°C under microaerophilic conditions. Whole cells were harvested and washed three times in 0.01 M Tris buffer (pH 7.5). The final pellet was suspended in a minimal amount of the 0.01 M Tris buffer, the protein concentration was determined by the Coomassie blue reaction (5), the concentration of cells was adjusted to 3.5 mg of protein per ml, and the cell suspension was stored at −70°C. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of whole cell preparations was performed by a modification of the method of Laemmli (14) in 0.8-mm-thick slab gels. The stacking gel contained 6% acrylamide. The separating gel was a 10 to 15% linear acrylamide gradient. SDS was not included in either the stacking or separating gel. Whole cell

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TABLE 1. *C. coli* strains used in this study

C. coli strain[a]	Source	Serogroup[b]	Biotype[b]	Geographic area of isolation	Meat packing establishment no.
C-602S	Swine	20	I	Ontario, Canada	
C-602R	Swine	20	I	Ontario, Canada	
C-793-1	Cattle	20	I	Ontario, Canada	1
C-793-2	Cattle	20	I	Ontario, Canada	1
C-793-3	Cattle	20	I	Ontario, Canada	2
C-793-4	Cattle	20	I	Ontario, Canada	3
C-793-5	Cattle	20	I	Ontario, Canada	4
C-793-6	Cattle	20	I	Ontario, Canada	5
C-793-7	Cattle	20	I	Ontario, Canada	6
C-793-8	Cattle	20	I	Ontario, Canada	6
C-793-9	Cattle	20	I	Ontario, Canada	7
C-793-10	Cattle	20	I	Ontario, Canada	8
C-264	Human	12	I	British Columbia, Canada	
C-699	Chicken	21	I	Ontario, Canada	
C-152-1	Cattle	26	I	Ontario, Canada	8
C-152-2	Cattle	26	I	Ontario, Canada	6
C-152-6	Cattle	29,55	I	Ontario, Canada	6
CS-501	Swine	24	I	Manitoba, Canada	
CS-589	Swine	38	I	Manitoba, Canada	

[a] Strains C-602S, C-602R, C-264, and C-699 were from the collection of H. Lior, and strains CS-501 and CS-589 were from the Animal Pathology Laboratory, Agriculture Canada, Winnipeg, Manitoba, Canada.
[b] Scheme of Lior (16, 17).

suspensions (10 to 15 μl) were placed in an equal volume of reducing buffer containing 1.25% (wt/vol) SDS, 1.25% (vol/vol) 2-mercaptoethanol, 12.5% (vol/vol) glycerol, 0.0625 M tris base (pH 6.8), and 0.00125% (wt/vol) bromophenol blue. Samples were boiled at 100°C for 5 min, and 10.5 μg of protein was applied to each lane. Electrophoresis was done at 20 mA constant current until the tracking dye reached the bottom of the separating gel. Protein profiles were visualized with Coomassie brilliant blue R 250. LPS profiles were detected by the silver stain method of Tsai and Frasch (28). Electrophoresis chemicals [acrylamide, *N,N'*-methylene bisacrylamide, tris(hydroxymethyl)aminomethane, glycine, SDS, and *N,N,N',N'*-tetramethylethylenediamine] and protein molecular weight standards (lysozyme, 14,400 subunit molecular weight [MW]; soybean trypsin inhibitor, 21,500 MW; carbonic anhydrase, 31,000 MW; ovalbumin, 45,000 MW; bovine serum albumin, 66,200 MW; phosphorylase B, 92,500 MW) were purchased from Bio-Rad (Bio-Rad Laboratories Canada Ltd., Mississauga, Ontario, Canada).

Plasmid DNA isolation. Bacteria were grown as described above for SDS-PAGE. The cells from one plate were suspended in 0.05 M Tris–0.02 M EDTA (pH 8.0; TE buffer), harvested by centrifugation, and washed once with TE buffer. Plasmids were extracted by the rapid alkaline-SDS procedure of Dillon et al. (8). This procedure is a modification of the techniques of Birnboim and Doly (2), Casse et al. (7), and Kado and Liu (11). The cells were treated for 30 min with 1 mg of lysozyme per ml and then lysed for 30 min with 1.5% SDS in TE buffer (pH 12.4). The mixture was neutralized with 3 M sodium acetate (pH 4.8) and placed on ice. After centrifugation, the supernatant was extracted with phenol. The DNA in the aqueous phase was precipitated in ethanol at −20°C overnight.

Plasmid DNA electrophoresis. The samples were subjected to horizontal 0.5% agarose gel electrophoresis for 3.5 h at 100 V in Tris-borate-EDTA buffer. Molecular size standards included were Tp 124, 120 megadaltons (MDa; a megadalton is equivalent to approximately 1.66×10^{-21} kg); R6K, 26 MDa; pSC201, 6 MDa; ColE1, 4.2 MDa; and pBR322, 2.6 MDa. The gels were then stained with ethidium bromide, and the DNA bands were visualized by transillumination with short (300-nm) UV light. Photographs were taken with a UV filter (Kodak Wratten gelatin filter no. 23A).

RESULTS

Isolation of cephalothin-susceptible *C. coli*. Fifty-seven thermophilic campylobacters were isolated from 92 beef carcasses taken from 16 randomly selected slaughter establishments in southern Ontario. Thirteen strains were identified as *C. coli*, and 44 strains were identified as *C. jejuni*. Of the 13 *C. coli* isolates, 10 (77%; C-793-1 to C-793-10) were susceptible to cephalothin. These were isolated from neck muscles and one gall bladder of nine slaughter cattle from

TABLE 2. Antimicrobic susceptibility patterns of *C. coli* strains

C. coli strain	Pattern[a] for the indicated antimicrobial disk (μg)						
	Cephalothin (30)	Tetracycline (30)	Erythromycin (15)	Clindamycin (2)	Streptomycin (10)	Sulfamethoxazole-trimethoprim (25)	Metronidazole (5)
C-602S	S	S	S	S	R	R	S
C-602R	R	S	S	S	R	R	S
C-793-1	S	S	S	S	R	R	S
C-793-2	S	S	S	S	R	R	S
C-793-3	S	S	S	S	R	R	S
C-793-4	S	S	S	S	R	R	S
C-793-5	S	S	S	S	R	R	S
C-793-6	S	S	S	S	R	R	S
C-793-7	S	S	S	S	R	R	S
C-793-8	S	S	S	S	R	R	S
C-793-9	S	S	S	S	R	R	S
C-793-10	S	S	S	S	R	R	S
C-264	S	S	S	S	S	R	S
C-699	R	S	S	S	S	R	S
C-152-1	R	S	S	S	S	S	S
C-152-2	R	I	S	S	S	S	S
C-152-6	R	I	S	S	R	S	S
CS-501	R	R	R	R	R	R	R
CS-589	I	S	S	S	CR	C	C

[a] S, Susceptible; R, resistant; I, intermediate; C, contaminated. For an interpretation of the susceptible, intermediate, or resistant pattern, see reference 1.

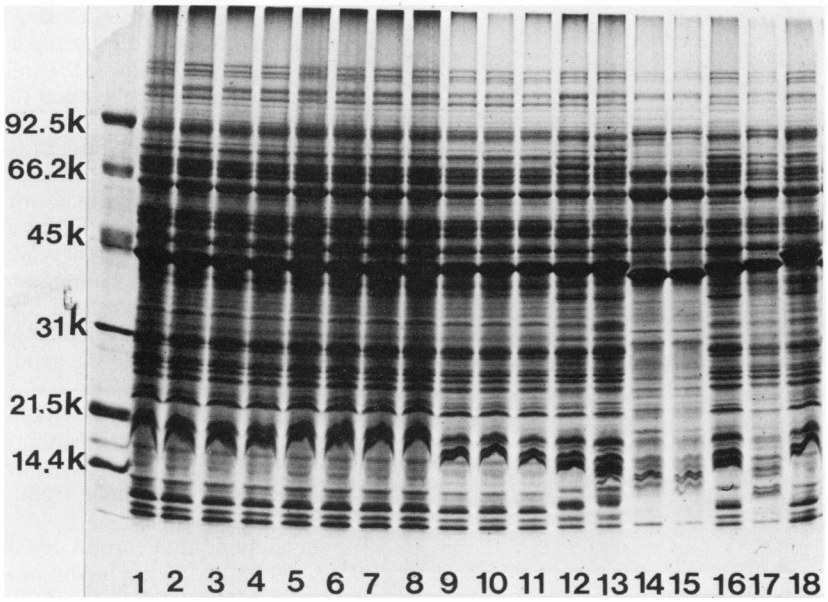

FIG. 1. SDS-PAGE of *C. coli* whole cells stained with Coomassie blue to visualize proteins. Lanes: 1, *C. coli* C-602S; 2 to 11, *C. coli* 793-1 to C-793-10, respectively; 12, *C. coli* C-264; 13, *C. coli* C-699; 14, *C. coli* C-152-1; 15, *C. coli* C-152-2; 16, *C. coli* 152-6; 17, *C. coli* CS-501; 18, *C. coli* CS-589. Molecular weight markers (10^3) appear to the left.

eight establishments. (Although care was taken in processing neck muscle and gall bladder samples, surface contamination could not be excluded.) All 10 strains were catalase and oxidase positive and grew at 37 and 43°C but not at 25°C. Strains C-793-1 and C-793-2 were isolated from the neck muscles and gall bladder of the same animal.

Antimicrobial susceptibility testing. The antimicrobial susceptibility patterns of the strains included in this study are shown in Table 2. Twelve strains were susceptible to cephalothin, and 11 of these (strains C-793-1 to C-793-10 and C-602S) had identical susceptibility patterns. The pattern of the other cephalothin-susceptible strain, C-264, differed only in susceptibility to streptomycin. All 19 strains were inhibited by nalidixic acid, gentamicin, and ampicillin (data not shown).

SDS-PAGE patterns. The SDS-PAGE whole cell protein profiles of 18 strains are shown in Fig. 1. The patterns of *C. coli* C-602S and C-793-1 to C-793-10 were very similar, with a single major protein band migrating at approximately 45,000. Strains C-264, C-699, and C-152-6 had similar profiles but could be distinguished from C-602S and C-793-1 to C-793-10 and from each other by differences in minor bands, particularly those in the molecular weight range of 60,000 to 92,000. Greater differences were observed between the patterns of *C. coli* C-152-1, C-152-2, CS-501, and CS-589 and the other *C. coli* strains, including migration distance of the major protein band and presence and relative staining intensity of minor bands. The protein profile of strain C-152-1 was very similar to that of C-152-2 but different from that of C-152-6.

Silver staining of whole cell lysates (Fig. 2) indicated the presence of fast-migrating, low-molecular-weight LPS in the 18 strains. The absence of detectable high-molecular-weight LPS bands indicated that O polysaccharide chains were either absent or present in quantities too low to detect. The LPS profiles of *C. coli* C-602S and 793-1 to 793-10 were very similar and could be distinguished from those of the other strains.

The protein and LPS profiles of *C. coli* C-602S and C-602R (Fig. 3) were very similar.

Plasmid profiles. The number and molecular sizes of the plasmids from the 19 strains are shown in Table 3. The profiles of *C. coli* C-602S, C-602R, and C-793-1 to C-793-10 were identical to each other and different from each of the other strains studied. Each contained two low-molecular-size plasmids of 2.4 and 2.8 MDa. *C. coli* CS-589 and C-264 also contained low-molecular-size plasmids of 2.2 and 2.5 MDa and 6.0 MDa, respectively, as well as a larger-molecular-size plasmid of 26 MDa. The other *C. coli* strains each contained a single large-molecular-size plasmid.

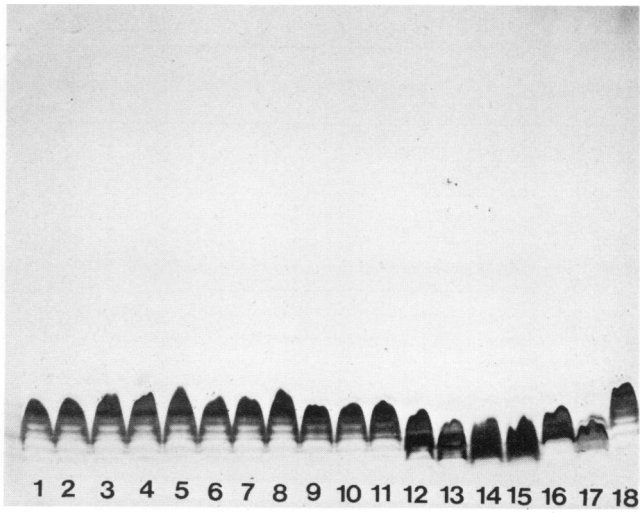

FIG. 2. SDS-PAGE of *C. coli* whole cell LPS profiles detected by the silver stain method of Tsai and Frasch (28). Lanes are as described in the legend to Fig. 1.

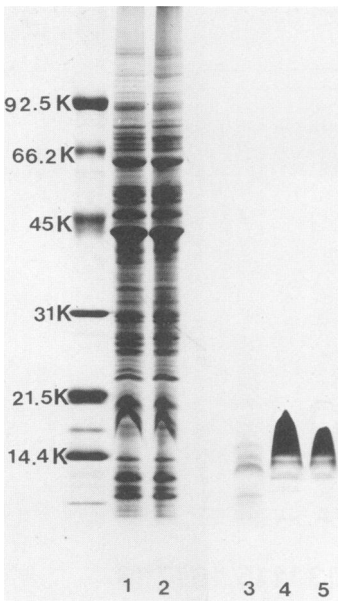

FIG. 3. SDS-PAGE of *C. coli* C-602R and C-602S. Lanes: 1, *C. coli* C-602R; 2, *C. coli* C-602S; 3, purified LPS from *Salmonella typhimurium*; 4, *C. coli* C-602R; 5, *C. coli* C-602S. Lanes 1 and 2 were stained with Coomassie blue, and lanes 3, 4, and 5 were stained by using the method of Tsai and Frasch (28). Molecular weight markers (10^3) appear to the left.

DISCUSSION

C. coli and *C. jejuni* are generally considered to be resistant to cephalothin (6, 12). However, in the present study 10 of 13 or 77% of the *C. coli* isolates from slaughter cattle were susceptible to cephalothin, as indicated by a clear zone with a diameter of 22 to 29 mm around a 30-μg cephalothin disk. The recovery of a high percentage of cephalothin-susceptible *C. coli* isolates from a survey of slaughter cattle suggested that such strains are more common in nature than previously reported (6, 13, 23, 29). Because cephalothin is considered to be ineffective against *C. coli* and *C. jejuni* and inhibitory for other gram-negative intestinal organisms, it has been included in the selective isolation media of Butzler (15) and Blaser et al. (3). An enrichment procedure developed to recover small numbers of campylobacters from foods (9) also incorporates the cephalothin-containing medium of Blaser et al. Cephalothin-susceptible strains of *C. coli* cannot be recovered by using these media. The enrichment broth of Rosef (24) and supplemented Mueller-Hinton blood agar, neither of which contain cephalothin but both of which incorporate vancomycin, polymyxin B, and trimethoprim, were used in the present study. Several standard identification schemes (13, 25) include cephalothin resistance as a key characteristic to differentiate species within the genus *Campylobacter*. Cephalothin-susceptible *C. coli* and *C. jejuni* isolates could be misidentified by using these schemes. The same identification schemes (13, 25) also consider most *C. coli* and *C. jejuni* isolates to be susceptible to nalidixic acid. However, Taylor et al. (26) reported a high frequency of nalidixic acid-resistant *C. jejuni*.

TABLE 3. Molecular sizes of *C. coli* plasmids

C. coli strain	Plasmid molecular size(s) (MDa)
C-602S	2.4, 2.8
C-602R	2.4, 2.8
C-793-1	2.4, 2.8
C-793-2	2.4, 2.8
C-793-3	2.4, 2.8
C-793-4	2.4, 2.8
C-793-5	2.4, 2.8
C-793-6	2.4, 2.8
C-793-7	2.4, 2.8
C-793-8	2.4, 2.8
C-793-9	2.4, 2.8
C-793-10	2.4, 2.8
C-264	6.0, 26
C-699	68
C-152-1	27
C-152-2	27
C-152-6	11
CS-501	26
CS-589	2.2, 2.5, 26

We recommend that careful consideration be given to the use of cephalothin in enrichment or selective isolation media and as a key diagnostic character for *C. coli*.

SDS-PAGE was used in this study to further characterize these organisms in terms of protein and LPS profiles. Such techniques have been used to study outer membrane proteins (4, 18), LPS (19, 22), and other components (21, 30) of various *Campylobacter* spp. Blaser et al. (3) and Logan and Trust (19) observed that with outer membrane and LPS profiles, respectively, there are strain-to-strain differences within the same species, particularly with regard to minor bands. In the present study, protein and LPS profiles of 11 cephalothin-susceptible *C. coli* strains were very similar, and antimicrobial susceptibility patterns were identical. These findings suggest that the latter 11 strains represent a *C. coli* clone which is susceptible to cephalothin. All 11 strains were serogroup 20 in the Lior scheme, and 10 were isolated from slaughter cattle at eight geographically distinct meat packing plants in southern Ontario. *C. coli* C-264, also cephalothin susceptible but different from the other 11 strains in terms of protein and LPS profiles, was isolated in a distant province (British Columbia). The latter strain was serogroup 12 in the Lior scheme and was the only one of the 12 cephalothin-susceptible strains which was not resistant to streptomycin.

Plasmid profiles are useful in epidemiological studies (27). Plasmids were detected in all 19 strains in this study. The profiles of the 10 cephalothin-susceptible *C. coli* strains from slaughter cattle and strain C-602S were identical but different from those of the other strains, again suggesting that these 11 strains represent a clone of *C. coli*. The similarity of the profiles of the cephalothin-susceptible strain, C-602S, and the cephalothin-resistant strain, C-602R, suggests that cephalothin resistance is not plasmid mediated.

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