2005

Antimicrobial resistance patterns of Campylobacter from feedlot cattle

M. D. Englen
USDA-ARS

R. J. Fedorka-Cray
USDA-ARS

S. R. Ladely
USDA-ARS

D. A. Dargatz
USDA-APHIS

Journal of Applied Microbiology 2005, **99,** 285–291 doi:10.1111/j.1365-2672.2005.02609.x

Antimicrobial resistance patterns of *Campylobacter* from feedlot cattle*

M.D. Englen[1], P.J. Fedorka-Cray[1], S.R. Ladely[1] and D.A. Dargatz[2]

[1]USDA-ARS, Bacterial Epidemiology and Antimicrobial Resistance Research Unit, Richard B. Russell Agricultural Research Center, Athens, GA, USA, and [2]USDA-APHIS, Centers for Epidemiology and Animal Health, Ft Collins, CO, USA

2004/0964: received 19 August 2004, revised 24 January 2004 and accepted 28 January 2004

ABSTRACT

M.D. ENGLEN, P.J. FEDORKA-CRAY, S.R. LADELY AND D.A. DARGATZ. 2005.

Aims: This study examined 448 *Campylobacter* strains isolated in 1999 and 2000 from US feedlot cattle for resistance to 12 antimicrobials.

Methods and Results: Isolates were tested for antimicrobial susceptibility using the E-test method. Approximately 60% ($n = 267$) were resistant to one or more antimicrobials, and 19·6% ($n = 88$) were resistant to two or more antimicrobials. Of the *Campylobacter jejuni* isolates, 49·1% ($n = 187$) were resistant to tetracycline, 10·2% ($n = 39$) were resistant to nalidixic acid, 8·4% were resistant to trimethoprim/sulfamethoxazole, and 1·8% ($n = 7$) were resistant to ciprofloxacin. Resistance to any of the other eight antimicrobials was 1·3% or less, but 14·4% ($n = 55$) were resistant to two or more antimicrobials. In the *Campylobacter coli* group, 65·7% ($n = 44$) were resistant to tetracycline, 52·2% ($n = 35$) were resistant to trimethoprim/sulfamethoxazole, 22·4% ($n = 15$) were resistant to nalidixic acid, and 9·0% ($n = 6$) were resistant to ciprofloxacin. Resistance to any of the remaining eight antimicrobials was 3·0% or less, although 49·3% ($n = 33$) were resistant to two or more antimicrobials.

Conclusions: Although antimicrobials are widely used in US feedlot cattle production, our results demonstrate generally low levels of resistance to a broad range of commonly used antimicrobials relative to other recent studies.

Significance and Impact of the Study: Resistance data on *Campylobacter* isolated from this major US livestock commodity is lacking. This overview enhances current knowledge and provides a basis for further studies.

Keywords: antimicrobials, *Campylobacter coli*, *Campylobacter jejuni*, cattle, resistance.

INTRODUCTION

Campylobacter is recognized as a major cause of acute bacterial gastroenteritis in humans worldwide (Friedman *et al.* 2000). Clinical signs include abdominal pain, fever, malaise, nausea, vomiting and diarrhoea (Skirrow and Blaser 2000). Most patients recover in less than a week, but up to 20% may relapse or experience prolonged or severe illness requiring antibiotic therapy. Two species of *Campylobacter*, *C. jejuni* and *C. coli*, are most frequently isolated from cases of human infection (Engberg *et al.* 2000).

A number of studies have investigated the epidemiology of *Campylobacter* in poultry breeder and broiler operations (van de Giessen *et al.* 1992; Humphrey *et al.* 1993; Jacobs-Reitsma 1995, 1997, 2000; Pearson *et al.* 1996), and poultry products are considered to be a main source of *Campylobacter* infections in humans (Deming *et al.* 1987; Doyle and Jones 1992). Prevalence studies have also shown that *Campylobacter* is a common commensal in cattle (Garcia *et al.* 1985; Manser and Dalziel 1985; Stanley *et al.* 1998; Wesley *et al.* 2000). Accordingly, beef and dairy products (especially raw milk) can be a source of human *Campylobacter* infection (Stern 1992; Headrick and Tollefson 1998).

*The mention of trade names or commercial products in this manuscript is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

Correspondence to: Mark D. Englen, USDA, Agricultural Research Service, Bacterial Epidemiology and Antimicrobial Resistance Research Unit, Richard B. Russell Agricultural Research Center, 950 College Station Road, Athens, GA 30605-2720, USA (e-mail: menglen@saa.ars.usda.gov).

The emergence of strains of *Campylobacter* and other food-borne bacterial pathogens resistant to antimicrobials used to treat human disease has provoked controversy over the use of antimicrobials in food animal production, including extensive examination of its effects on antimicrobial resistance in these organisms (Khachatourians 1998; Teuber 2001). Antimicrobial resistance in *Campylobacter* isolated from the poultry production environment has been well documented in studies from around the world (Jacobs-Reitsma *et al.* 1994; Chuma *et al.* 2001; Heuer *et al.* 2001; Van Looveren *et al.* 2001). In contrast, while cattle have been included in multi-species investigations (Aarestrup *et al.* 1997; Piddock *et al.* 2000), reports are lacking on antimicrobial resistance in *Campylobacter* isolated from cattle.

This study examined resistance to 12 common antimicrobials among a geographically diverse group of *Campylobacter* strains isolated in 1999 and 2000 from US feedlot cattle.

MATERIALS AND METHODS

Study design, and bacterial isolation and identification

The *Campylobacter* isolates used were collected as part of the 1999 National Animal Health Monitoring System study of the health and management of US feedlot cattle (Feedlot '99; USDA 2000). The study focused on feedlots with 1000 head or more capacity as these facilities held over 80% of US cattle as of February 1999. Faecal samples were obtained from 73 feedlots in 11 major US feedlot states: California, Colorado, Idaho, Iowa, Kansas, Nebraska, New Mexico, Oklahoma, South Dakota, Texas and Washington. Samples were collected during a 1-year period (October 1999 through September 2000) from pen floors. For each feedlot, 25 samples were collected from three pens. Feedlots were sampled twice during the 1-year collection period. Samples were shipped for overnight delivery to the USDA-ARS-ARRU laboratory in Athens (GA, USA) for isolation of *Campylobacter* and *Salmonella*.

For *Campylobacter* isolation, faecal samples were diluted 1 : 4 and 1 : 40 in phosphate-buffered saline. One hundred-microlitre aliquots of each dilution were spread uniformly on duplicate Campy-Cefex plates (Stern *et al.* 1992). The plates were placed in zip-top bags and incubated microaerobically (5% O_2, 10% CO_2 and 85% N_2) for 48 h at 42°C. *Campylobacter* was presumptively identified from microscope wet mounts of cells using phase contrast optics at 100×.

For the present study, a total of 448 isolates comprising 43·5% (448 of 1029) of all the Feedlot '99 *Campylobacter* strains were randomly selected from among the frozen stock Feedlot '99 culture collection. Isolates included were derived from 67·1% (49 of 73) of all feedlots sampled with some isolates from each of the 11 states where sampling occurred. Cultures were streaked onto Campy-Cefex plates, and incubated microaerobically for 48 h at 42°C. Isolated colonies were picked from these plates, streaked again onto Campy-Cefex plates, and incubated microaerobically for 48 h at 42°C. Isolated colonies from these plates were streaked onto trypticase soya agar plates containing 5% sheep's blood (BA plates; B-D Biosciences, Sparks, MD, USA), and incubated as before for 48 h at 42°C. The BA plates were used for isolate testing and to prepare additional frozen stock cultures. Isolates were identified using the *Campylobacter* BAX® PCR (DuPont Qualicon, Wilmington, DE, USA), a multiplex assay specific for *C. coli* and *C. jejuni*. The assay was performed according to the manufacturer directions as previously described (Englen and Fedorka-Cray 2002).

Antimicrobial susceptibility testing

Isolates were tested for antimicrobial susceptibility using the E-test (AB-Biodisk, Piscataway, NJ, USA) according to the manufacturer directions. This method has been adopted by the National Antimicrobial Resistance Monitoring System (NARMS) for susceptibility testing of *Campylobacter*, in part, because it facilitates screening large numbers of isolates. It has been shown to give results comparable with other methods such as agar dilution for *Campylobacter* (Luber *et al.* 2003; Oncul *et al.* 2003). In brief, 150 mm Mueller–Hinton + 5% lysed horse blood plates (B-D Biosciences) were inoculated with 100 μl of a cell suspension equivalent to 1·0 McFarland standard. The inoculum was swabbed evenly across the entire plate surface. E-test strips were brought to room temperature from −20°C storage before use. Four strips were laid at 90° angles onto each plate. The plates were placed in zip-top bags and incubated microaerobically for 48 h at 42°C. Following incubation, the point at which the zone of growth inhibition intersected the strip was read as the minimum inhibitory concentration (MIC) of the antimicrobial in $\mu g\ ml^{-1}$. Quality control ATCC strains *C. jejuni* 33560, *Escherichia coli* 25922 and *Staphylococcus aureus* 25923 were tested biweekly to confirm susceptibility to all the antimicrobials. The antimicrobials used in the study and the resistance breakpoints (MICs) were: amoxicillin/clavulanic acid, ≥32; azithromycin, ≥2; cefepime, ≥32; chloramphenicol, ≥32; ciprofloxacin, ≥4; clindamycin, ≥4; erythromycin, ≥8; gentamicin, ≥16; imipenem, ≥16; nalidixic acid, ≥32; tetracycline, ≥16; trimethoprim/sulfamethoxazole, ≥32. With the exception of cefepime, the MICs for the respective resistance breakpoints were those used by NARMS as reported in the US Centers for Disease Control NARMS 2001 Annual Report (http://www.cdc.gov/narms/annuals.htm). For cefepime, the

E-test manufacturer's recommended resistance breakpoint for anaerobes was used.

Data analysis

The antimicrobial resistance data was analysed using the proportional hazard model (PHREG) in the SAS statistical program (SAS Institute, Cary, NC, USA). This method performs conditional logistic regression and provides adjustment for the fact that the 448 isolates (i.e. observations) were not entirely independent. Rather, the isolates were obtained from 73 different feedlots, with some imbalance between the proportion of *C. coli* and *C. jejuni* observed in each feedlot. This method and its applications are discussed in detail by Allison (1999).

RESULTS

Antimicrobial resistance

Approximately 60% ($n = 267$) of the 448 isolates included in the study were resistant to one or more antimicrobials. The percentages by species of isolates resistant to the individual antimicrobials are shown in Table 1. The greatest level of resistance observed was to tetracycline, at 51·6% ($n = 231$) for the test group as a whole. Resistance to trimethoprim/sulfamethoxazole was next highest, at 15·0% ($n = 67$) overall, followed by nalidixic acid at 12·1% ($n = 54$). The isolates showed much less resistance to the remaining nine antimicrobials. Among these, ciprofloxacin resistance was the greatest, although reaching only 2·9% ($n = 13$). However, a number of differences in antimicrobial resistance were observed between species (Table 1). In particular, resistance in the *C. coli* group to nalidixic acid was more than twice as high as for the *C. jejuni* group (22·4%, $n = 15$ *vs* 10·2%, $n = 39$; $P < 0·05$), and five times higher for ciprofloxacin, compared with the *C. jejuni* strains (9·0%, $n = 6$ *vs* 1·8%, $n = 7$; $P < 0·05$). Similarly, resistance to trimethoprim/sulfamethoxazole was observed in 52·2% of the *C. coli* ($n = 35$) isolates, more than six times that of *C. jejuni* (8·4%, $n = 32$; $P < 0·05$). The *C. coli* isolates were also more resistant to tetracycline than the *C. jejuni* strains (65·7%, $n = 44$ *vs* 49·1%, $n = 187$), although this difference was not significant at the 5% level.

Table 1 Percentage of *Campylobacter* isolates resistant to antimicrobials by species

Antimicrobial	*C. jejuni* ($n = 381$)	*C. coli* ($n = 67$)	Total ($n = 448$)
Amoxicillin/clavulanic acid	0	0	0
Azithromycin	0·8 ($n = 3$)	1·5 (1)	0·9 (4)
Cefepime	1·3 (5)	1·5 (1)	1·3 (6)
Chloramphenicol	1·0 (4)	3·0 (2)	1·3 (6)
Ciprofloxacin	1·8 (7)	9·0* (6)	2·9 (13)
Clindamycin	1·0 (4)	1·5 (1)	1·1 (5)
Erythromycin	0·5 (2)	3·0 (2)	0·9 (4)
Gentamicin	0·25 (1)	0	0·2 (1)
Imipenem	0·25 (1)	1·5 (1)	0·4 (2)
Nalidixic Acid	10·2 (39)	22·4* (15)	12·1 (54)
Tetracycline	49·1 (187)	65·7 (44)	51·6 (231)
Trimethoprim/sulfamethoxazole	8·4 (32)	52·2* (35)	15·0 (67)

*Significantly higher resistance ($P < 0·05$) compared with *C. jejuni*.

Multiple resistance

Of the 448 isolates tested, 19·6% ($n = 88$) were resistant to two or more antimicrobials. A significantly higher percentage of the *C. coli* strains (49·3%, $n = 33$; $P < 0·05$) belonged to this group compared with the *C. jejuni* isolates (14·4%, $n = 55$). The majority of these 88 strains (71·6%, $n = 63$) were resistant to just two antimicrobials (Table 2). Of the 63 isolates resistant to two antimicrobials, 49·2% ($n = 31$; 14 *C. jejuni* and 17 *C. coli*) combined resistance to tetracycline and trimethoprim/sulfamethoxazole. The next most common pattern was resistance to nalidixic acid and tetracycline, found in 39·7% ($n = 25$; 24 *C. jejuni* and one *C. coli*) of these strains. The remaining four patterns of double resistance were comprised of three or fewer isolates.

Table 2 Resistance patterns of *Campylobacter* isolates resistant to two or more antimicrobials ($n = 88$)

No. of resistances	Resistance pattern	No. of isolates with resistance pattern	
		C. jejuni	*C. coli*
2	TC TS	14	17
2	NA TC	24	1
2	CI NA	2	1
2	PM TC	2	–
2	CM TC	1	–
2	EM TC	1	–
3	CI NA TC	5	1
3	NA TC TS	2	5
3	CI NA TS	–	1
3	PM TC TS	1	–
4	CI NA TC TS	–	3
4	AZ CL CM TC	2	–
4	EM IP TC TS	–	1
4	AZ CL EM TC	–	1
4	CM NA TC TS	–	1
4	CL NA TC TS	–	1
7	AZ CL CM EM NA PM TC	1	–

AZ, azithromycin; CL, chloramphenicol; CI, ciprofloxacin; CM, clindamycin; EM, erythromycin; IP, imipenem; NA, nalidixic acid; PM, cefepime; TC, tetracycline; TS, trimethoprim/sulfamethoxazole.

Resistance to three or more antimicrobials was found in 28·4% ($n = 25$) of the 88 multi-resistant isolates or 5·6% of all isolates (Table 2). Significantly, seven times more *C. coli* were found in this group compared with the *C. jejuni* strains (20·9%, $n = 14$ *vs* 2·9%, $n = 11$; $P < 0·05$). Among the 11 resistance patterns found for those isolates resistant to three or more antimicrobials, two were more common. Most frequently observed was the combination of nalidixic acid, tetracycline and trimethoprim/sulfamethoxazole resistance, found in 28% ($n = 7$) of these 25 strains (Table 2). Resistance to ciprofloxacin, nalidixic acid and tetracycline was observed in 24% ($n = 6$) of this group. Moreover, a single *C. jejuni* isolate was found to be resistant to seven antimicrobials.

DISCUSSION

The use of antimicrobials in food animal production has become a source of great controversy in recent years. Concerns over the emergence of bacterial pathogens resistant to antimicrobials commonly used to treat infections in humans, and the potential transfer of resistant pathogenic strains from food products to humans has led to changes in antimicrobial usage in livestock and poultry production worldwide (Aarestrup and Wegner 1999; McEwen and Fedorka-Cray 2002). Monitoring susceptibility patterns to important antimicrobials among pathogens isolated from food animal production sources is an essential component in assessing risk associated with antimicrobial use in agriculture. Antimicrobial susceptibility was examined in *Campylobacter* isolated from US feedlot cattle. To date, this livestock group has received relatively little attention in antimicrobial resistance-monitoring studies.

A high level of resistance was observed to tetracycline, exceeding 51% for the isolate group as a whole (Table 1). This finding was not surprising as tetracyclines are commonly used as injectable therapeutics for respiratory disease, and as feed or water additives during the feedlot stage of beef cattle production in the USA (USDA 2000). Among the *Campylobacter* species, tetracycline resistance was noticeably higher in *C. coli*, reaching nearly 66% compared with 49·1% for *C. jejuni*. These numbers are substantially higher than the average reported in a recent multi-nation European study (Bywater *et al.* 2004) for tetracycline resistance in *C. coli* (41·2%) and *C. jejuni* (19·9%) from cattle. This may be a consequence of more limited overall use of this drug in the countries that provided the *Campylobacter* isolates (Germany, Italy and the UK).

In contrast, although it has been estimated that only *c.* 0·3% of the cattle represented by the Feedlot '99 study received sulfonamides in the feed or water, the overall resistance to trimethoprim/sulfamethoxazole was 15·0%. A study by Karmali *et al.* (1981) reported very high levels of resistance to trimethoprim and sulfamethoxazole (100 and 85% of the isolates, respectively, at 32 μg ml^{-1}) in *C. jejuni* from human faecal isolates. *Campylobacter jejuni* has subsequently come to be regarded as endogenously resistant to trimethoprim (Gibreel and Sköld 1998). Nonetheless, resistance to trimethoprim/sulfamethoxazole among *C. jejuni* in our study was only 8·4% ($n = 32$) compared with 52·2% ($n = 35$) for the *C. coli* strains (Table 1). Whether this indicates a greater propensity for *C. coli* to acquire resistance to trimethoprim/sulfamethoxazole compared with *C. jejuni* remains to be determined, although it is again reflective of the generally higher levels of resistance found in *C. coli* (this study).

The fluoroquinolone enrofloxacin is approved for use in the USA for the treatment of bovine respiratory disease in cattle, and *c.* 32% of the feedlots in the Feedlot '99 study reported using this antimicrobial therapeutically in some cattle (USDA 2000). Still, resistance to ciprofloxacin, the active metabolite of enrofloxacin, was only 2·9% overall, although in *C. coli* resistance was 9·0% (Table 1). Feedlot cattle are administered enrofloxacin as a single intramuscular injection (http://www.fda.gov/cvm/greenbook.html). Concentrations of an injected fluoroquinolone reaching the gut probably remain quite low. Resistance to ciprofloxacin was one-fourth that of nalidixic acid (2·9 and 12·1% respectively), a finding similar to that described by Aarestrup *et al.* (1997) for quinolone resistance in *Campylobacter* from cattle. Among species, nalidixic acid resistance in *C. coli* was more than twice that of *C. jejuni* (22·4% *vs* 10·2%). In contrast, Bywater *et al.* (2004) found relatively high resistance to ciprofloxacin in *C. coli* and *C. jejuni* from cattle (23·5 and 13·5% respectively), with comparable levels of resistance to nalidixic acid.

A primary mechanism of resistance to quinolones in *Campylobacter* involves discrete point mutations in the *gyrA* gene, which encodes DNA gyrase (a type II topoisomerase) (Drilca and Zhao 1997). In particular, the frequently observed substitution Thr86-Ile in *Campylobacter gyrA* is known to confer cross-resistance to both ciprofloxacin and nalidixic acid (Wang *et al.* 1993; Gibreel *et al.* 1998). Accordingly, 100% ($n = 13$) of the ciprofloxacin-resistant isolates were also resistant to nalidixic acid. However, 41 isolates (32 *C. jejuni* and nine *C. coli*), comprising 75·9% of the 54 quinolone-resistant strains found in this study, were resistant to nalidixic acid but did not show cross-resistance to ciprofloxacin. The increased prevalence of nalidixic acid resistance compared with ciprofloxacin was surprising, considering that the feedlot cattle in this study were never treated with nalidixic acid. In US broiler production, enrofloxacin has been administered orally to flocks in the drinking water to control and treat colibacillosis associated with *E. coli* infection (http://www.fda.gov/cvm/

greenbook.html). Similarly, higher levels of resistance to nalidixic acid compared with ciprofloxacin have been reported for the NARMS *Campylobacter* poultry isolates (http://www.ars-grin.gov/ars/SoAtlantic/Athens/arru/narms.html), the source of which is broiler carcasses. Bachoual *et al.* (2001) found that the Thr86-Ala *gyrA* substitution in *C. jejuni* resulted in resistance to nalidixic acid (MIC 64 μg ml^{-1}) but not to ciprofloxacin (MIC 2 μg ml^{-1}). This may explain the observed differences for ciprofloxacin and nalidixic acid resistance, although other mechanisms such as active efflux might also be involved.

Macrolides, in particular tilmicosin and tylosin, are also frequently used in injectable form as therapeutics, and tylosin was used as a feed or water additive on 20% of the feedlots represented in the Feedlot '99 study of health and management in US feedlots (USDA 2000). However, resistance did not exceed 1·1% for the test group overall to any of the three macrolide/lincosamide agents (azithromycin, clindamycin and erythromycin) included in this study (Table 1). Cross-resistance to these antimicrobials in strains resistant to tilmicosin or tylosin might be expected, as discrete point mutations in the 23S rRNA gene appear to be the primary mechanism involved in macrolide resistance in *Campylobacter* (Engberg *et al.* 2001; Vacher *et al.* 2003). Nonetheless, resistance to erythromycin, still considered a drug of choice for treating *Campylobacter* infection, was only 0·9% overall (Table 1). Moreover, macrolide resistance was far less than has been observed in *Campylobacter* from swine (Aarestrup *et al.* 1997; Sáenz *et al.* 2000; Van Looveren *et al.* 2001; Bywater *et al.* 2004), even though drugs of this class are commonly used in both cattle and swine production. This indicates that even relatively high usage of antimicrobials in particular class of food animals does not necessarily lead to high levels of resistance to related antimicrobials important in human medicine.

Although the use of chloramphenicol in livestock production has been banned for many years in the USA, a fluorinated derivative, florfenicol, is approved for use in treating respiratory disease in feedlot cattle. Of the feedlots represented in the Feedlot '99 study, 54% used florfenicol as a treatment for respiratory disease in some cattle, and 22·1% used florfenicol metaphylactically to control shipping fever in some cattle (USDA 2000). Cross-resistance to florfenicol and chloramphenicol has been reported in *E. coli* (White *et al.* 2000) and *Salmonella* (Bolton *et al.* 1999) resulting from the acquisition of a unique gene termed *flo*. Resistance to chloramphenicol in *Campylobacter* is most commonly mediated by chloramphenicol acetyltransferase which inactivates the drug by acetylation (Wang and Taylor 1990). This mechanism does not confer cross-resistance to florfenicol (Keyes *et al.* 2000). The lack of selective pressure by florfenicol would account for the low overall resistance (1·3%; Table 1) observed to chloramphenicol.

The majority of *Campylobacter* isolates show resistance to penicillins and narrow spectrum cephalosporins (Tajada *et al.* 1996; Treiber and Taylor 2000), but this activity rarely extends to the newer β-lactam agents. Consistent with this observation, resistance to imipenem, cefepime and amoxicillin/clavulanic acid did not exceed 1·3% (Table 1) although cephalosporins and penicillin/amoxicillin were used in 8–38% of feedlots to treat some cases of respiratory disease (USDA 2000).

Aminoglycosides, including gentamicin, are not generally used to treat beef cattle in the USA, due to, in part, the long duration of drug residues in some tissues. Not surprisingly, only a single isolate among the 448 *Campylobacter* strains included in this study was resistant to gentamicin.

Multiple resistance, including all isolates resistant to two or more antimicrobials, was found more than three times as often in the *C. coli* strains (49·3%; 33 of 67) compared with the *C. jejuni* isolates (14·4%; 55 of 381). Moreover, resistance to three or more antimicrobials, found in 5·6% (25 of 448) of all isolates (Table 2), was over seven times more common among the *C. coli* isolates (20·9%; 14 of 67) compared with *C. jejuni* (2·9%; 11 of 381). Multidrug resistance in *Campylobacter* has recently been reported to be associated with the expression of the *cmeABC* efflux system (Randall *et al.* 2003). While it may be speculated that this system is expressed more often in certain *Campylobacter* species such as *C. coli*, further work will be required to fully explain this observation. Among the isolates resistant to three or more antimicrobials, few patterns were apparent. However, we noted that ciprofloxacin resistance in this group was primarily associated with tetracycline resistance (Table 2). This association has been seen in previous studies, although the genetic basis has not been described (Piddock 1995; Gaunt and Piddock 1996). Resistance to macrolides among the multi-resistant isolates showed a mixture of patterns. Specifically, resistance to azithromycin and clindamycin or azithromycin and erythromycin was observed, as well as resistance to only clindamycin or erythromycin (Table 2). Only one strain in this group was resistant to azithromycin, clindamycin and erythromycin, an unusual *C. jejuni* isolate resistant to seven antimicrobials. Although the mechanisms involved in macrolide resistance in *Campylobacter* are not fully defined, cross-resistance to azithromycin, clindamycin and erythromycin might have been expected to be more commonly observed based on previous reports (Engberg *et al.* 2001; Vacher *et al.* 2003).

In conclusion, despite the widespread use of antimicrobials in US feedlot cattle production, generally low levels of antimicrobial resistance were observed in *Campylobacter* isolated from this livestock source. A recent review of European antimicrobial susceptibility surveillance results by Bywater (2004) also noted that *Campylobacter* isolates from cattle showed lower levels of resistance compared with

broiler and swine carcass isolates. Tetracycline resistance was an exception, although high levels of resistance to this drug are often found in *Campylobacter* from food production animals (e.g. Sáenz *et al.* 2000; Van Looveren *et al.* 2001; Bywater *et al.* 2004). Some of the differences reported on antimicrobial resistance in *Campylobacter* from various food animal sources may be attributed to the susceptibility test methods used (i.e. agar disk diffusion or agar dilution *vs* E-test), and to differences in actual drug use practices. Even so, obtaining antimicrobial resistance data from all food animal groups is essential to the development and management of rational antimicrobial use practices in food animal production.

ACKNOWLEDGEMENTS

The authors thank Rhonda Gantt, Sandra House, Leena Jain, Jennifer Murphy and Jodie Plumblee of the Bacterial Epidemiology and Antimicrobial Resistance Research Unit for excellent technical assistance. We also thank Dr Jaxk Reeves and Ms Jing Shen of the University of Georgia's Statistical Counseling Center for valuable assistance with the logistic regression analysis of the data set.

REFERENCES

Aarestrup, F.M. and Wegner, H.C. (1999) The effects of antibiotic usage in food animals on the development of antimicrobial resistance of importance for humans in *Campylobacter* and *Escherichia coli*. *Microbes Infect* **1**, 639–644.

Aarestrup, F.M., Nielsen, E.M., Madsen, M. and Engberg, J. (1997) Antimicrobial susceptibility patterns of thermophilic *Campylobacter* spp. from humans, pigs, cattle, and broilers in Denmark. *Antimicrob Agents Chemother* **41**, 2244–2250.

Allison, P.D. (1999) *Logistic Regression Using the SAS System: Theory and Application*, pp. 202–206. Cary, NC: BBU Press.

Bachoual, R., Ouabdesselam, S., Mory, F., Lascols, C., Soussy, C.-J. and Tankovic, J. (2001) Single or double mutational alterations of *gyrA* associated with flouroquinolone resistance in *Campylobacter jejuni* and *Campylobacter coli*. *Microb Drug Resist* **7**, 257–261.

Bolton, L.F., Kelley, L.C., Lee, M.D., Fedorka-Cray, P.J. and Maurer, J.J. (1999) Detection of multidrug-resistant *Salmonella enterica* serotype *typhimurium* DT104 based on a gene which confers cross-resistance to florfenicol and chloramphenicol. *J Clin Microbiol* **37**, 1348–1351.

Bywater, R. (2004) Veterinary use of antimicrobials and emergence of resistance in zoonotic and sentinel bacteria in the EU. *J Vet Med B* **51**, 361–363.

Bywater, R., Deluyker, H., Deroover, E., de Jong, A., Marion, H., McConville, M., Rowan, T., Shryock, T. *et al.* (2004) A European survey of antimicrobial susceptibility among zoonotic and commensal bacteria from food-producing animals. *J Antimicrob Chemother* **54**, 744–754.

Chuma, T., Ikeda, T., Maeda, T., Niwa, H. and Okamoto, K. (2001) Antimicrobial susceptibilities of *Campylobacter* strains isolated from broilers in the southern part of Japan from 1995 to 1999. *J Vet Med Sci* **63**, 1027–1029.

Deming, M.S., Tauxe, R.V., Blake, P.A., Dixon, S.E., Fowler, B.S., Jones, T.S., Lockamy, E.A., Patton, C.M. *et al.* (1987) *Campylobacter* enteritis at a university: transmission from eating chicken and from cats. *Am J Epidemiol* **126**, 526–534.

Doyle, M.P. and Jones, D.M. (1992) Food-borne transmission and antibiotic resistance of *Campylobacter jejuni*. In *Campylobacter jejuni: Current Status and Future Trends* ed. Nachamkin, I., Blaser, M.J. and Tompkins, L.S. pp. 45–48. Washington, DC: American Society for Microbiology.

Drilca, K. and Zhao, X. (1997) DNA gyrase, topoisomerase IV, and the 4-quinolones. *Microbiol Mol Biol Rev* **61**, 377–392.

Engberg, J., On, S.L.W., Harrington, C.S. and Gerner-Smidt, P. (2000) Prevalence of *Campylobacter*, *Arcobacter*, *Helicobacter*, and *Sutterella* spp. in human fecal samples as estimated by a reevaluation of isolation methods for campylobacters. *J Clin Microbiol* **38**, 286–291.

Engberg, J., Aarestrup, F.M., Taylor, D.E., Gerner-Smidt, P. and Nachamkin, I. (2001) Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: resistance mechanisms and trends in human isolates. *Emerg Infect Dis* **7**, 24–34.

Englen, M.D. and Fedorka-Cray, P.J. (2002) Evaluation of a commercial diagnostic PCR for the identification of *Campylobacter jejuni* and *Campylobacter coli*. *Lett Appl Microbiol* **35**, 353–356.

Friedman, C.R., Neimann, J., Wegner, H.C. and Tauxe, R.V. (2000) Epidemiology of *Campylobacter jejuni* Infections in the United States and other industrialized nations. In *Campylobacter*, 2nd edn. ed. Nachamkin, I. and Blaser, M.J. pp. 121–138. Washington, DC: American Society for Microbiology.

Garcia, M.M., Lior, H., Stewart, R.B., Ruckerbauer, G.M., Trudel, J.R. and Skljarevski, A. (1985) Isolation, characterization, and serotyping of *Campylobacter jejuni* and *Campylobacter coli* from slaughter cattle. *Appl Environ Microbiol* **49**, 667–672.

Gaunt, P.N. and Piddock, L.J.V. (1996) Ciprofloxacin resistant *Campylobacter* spp. in humans: an epidemiological and laboratory study. *J Antimicrob Chemother* **37**, 747–757.

Gibreel, A. and Sköld, O. (1998) High-level resistance to trimethoprim in clinical isolates of *Campylobacter jejuni* by acquisition of foreign genes (*dfr1* and *dfr9*) expressing drug-insensitive dihydrofolate reductases. *Antimicrob Agents Chemother* **42**, 3059–3064.

Gibreel, A., Sjögren, E., Kaijser, B., Wretlind, B. and Sköld, O. (1998) Rapid emergence of high-level resistance to quinolones in *Campylobacter jejuni* associated with mutational changes in *gyrA* and *parC*. *Antimicrob Agents Chemother* **42**, 3276–3278.

van de Giessen, A., Mazurier, S.I., Jacobs-Reitsma, W., Jansen, W., Berkers, P., Ritmeester, W. and Wernars, K. (1992) Study on the epidemiology and control of *Campylobacter jejuni* in poultry broiler flocks. *Appl Environ Microbiol* **58**, 1913–1917.

Headrick, M.L. and Tollefson, L. (1998) Food borne disease summary by food commodity. *Vet Clin North Am Food Anim Pract* **14**, 91–100.

Heuer, O.E., Pedersen, K., Andersen, J.S. and Madsen, M. (2001) Prevalence and antimicrobial susceptibility of thermophilic *Campylobacter* in organic and conventional broiler flocks. *Lett Appl Microbiol* **33**, 269–274.

Humphrey, T.J., Henley, A. and Lanning, D.G. (1993) The colonization of broiler chickens with *Campylobacter jejuni* – some epidemiological investigations. *Epidemiol Infect* **110**, 601–607.

Jacobs-Reitsma, W.F. (1995) *Campylobacter* bacteria in breeder flocks. *Avian Dis* **39**, 355–359.

Jacobs-Reitsma, W.F. (1997) Aspects of epidemiology of *Campylobacter* in poultry. *Vet Q* **19**, 113–117.

Jacobs-Reitsma, W.F. (2000) *Campylobacter* in the food supply. In *Campylobacter*, 2nd edn ed. Nachamkin, I. and Blaser, M.J. pp. 467–481. Washington, DC: American Society for Microbiology.

Jacobs-Reitsma, W.F., Koenraad, P.M., Bolder, N.M. and Mulder, R.W. (1994) In vitro susceptibility of *Campylobacter* and *Salmonella* isolates from broilers to quinolones, ampicillin, tetracycline, and erythromycin. *Vet Q* **16**, 206–208.

Karmali, M.A., de Grandis, S. and Fleming, P.C. (1981) Antimicrobial susceptibility of *Campylobacter jejuni* with special reference to resistance patterns of Canadian isolates. *Antimicrob Agents Chemother* **19**, 593–597.

Keyes, K., Hudson, C., Maurer, J.J., Thayer, S., White, D.G. and Lee, M.D. (2000) Detection of florfenicol resistance genes in *Escherichia coli* isolated from sick chickens. *Antimicrob Agents Chemother* **44**, 421–424.

Khachatourians, G.G. (1998) Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. *Can Med Assoc J* **159**, 1129–1136.

Luber, P., Bartelt, E., Genschow, E., Wagner, J. and Hahn, H. (2003) Comparison of broth microdilution, E test, and agar dilution methods for antibiotic susceptibility testing of *Campylobacter jejuni* and *Campylobacter coli*. *J Clin Microbiol* **41**, 1062–1068.

Manser, P.A. and Dalziel, R.W. (1985) A survey of *Campylobacter* in animals. *J Hyg* **95**, 15–21.

McEwen, S.A. and Fedorka-Cray, P.J. (2002) Antimicrobial use and resistance in animals. *Clin Infect Dis* **34** (Suppl. 3), S93–S106.

Oncul, O., Zarakolu, P., Oncul, O. and Gur, D. (2003) Antimicrobial susceptibility testing of *Campylobacter jejuni*: a comparison between Etest and agar dilution method. *Diagn Microbiol Infect Dis* **45**, 69–71.

Pearson, A.D., Greenwood, M.H., Feltham, R.K.A., Healing, T.D., Donaldson, J., Jones, D.M. and Colwell, R.R. (1996) Microbial ecology of *Campylobacter jejuni* in a United Kingdom chicken supply chain: intermittent common source, vertical transmission, and amplification by flock propagation. *Appl Environ Microbiol* **62**, 4614–4620.

Piddock, L.J.V. (1995) Quinolone resistance and *Campylobacter* spp. *J Antimicrob Chemother* **36**, 891–898.

Piddock, L.J.V., Ricci, V., Stanley, K. and Jones, K. (2000) Activity of antibiotics used in human medicine for *Campylobacter jejuni* isolated from farm animals and their environment in Lancashire, UK. *J Antimicrob Chemother* **46**, 303–306.

Randall, L.P., Ridley, A.M., Cooles, S.W., Sharma, M., Sayers, A.R., Pumbwe, L., Newell, D.G., Piddock, L.J.V. *et al.* (2003) Prevalence of multiple antibiotic resistance in 443 *Campylobacter* spp. isolated from humans and animals. *J Antimicrob Chemother* **52**, 507–510.

Sáenz, Y., Zarazaga, M., Lantero, M., Gastañares, M.J., Baquero, F. and Torres, C. (2000) Antibiotic resistance in *Campylobacter* strains isolated from animals, foods, and humans in Spain in 1997–1998. *Antimicrob Agents Chemother* **44**, 267–271.

Skirrow, M.B. and Blaser, M.J. (2000) Clinical aspects of *Campylobacter* infection. In *Campylobacter*, 2nd edn ed. Nachamkin, I. and Blaser, M.J. pp. 69–88. Washington, DC: American Society for Microbiology.

Stanley, K.N., Wallace, J.S., Currie, J.E., Diggle, P.J. and Jones, K. (1998) The seasonal variation of thermophilic campylobacters in beef cattle, dairy cattle, and calves. *J Appl Microbiol* **85**, 472–480.

Stern, N.J. (1992) Reservoirs for *Campylobacter jejuni* and approaches for intervention in poultry. In *Campylobacter jejuni: Current Status and Future Trends* ed. Nachamkin, I., Blaser, M.J. and Tompkins, L.S. pp. 49–60. Washington, DC: American Society for Microbiology.

Stern, N.J., Wojton, B. and Kwiatek, K. (1992) A differential-selective medium and dry ice-generated atmosphere for recovery of *Campylobacter jejuni*. *J Food Prot* **55**, 514–517.

Tajada, P., Gomez-Garces, J.-L., Alós, J.-I., Balas, D. and Cogollos, R. (1996) Antimicrobial susceptibilities of *Campylobacter jejuni* and *Campylobacter coli* to 12 β-lactam agents and combinations with β-lactamase inhibitors. *Antimicrob Agents Chemother* **40**, 1924–1925.

Teuber, M. (2001) Veterinary use and antibiotic resistance. *Curr Opin Microbiol* **4**, 493–499.

Treiber, C.A. and Taylor, D.E. (2000) Mechanisms of antibiotic resistance in *Campylobacter*. In *Campylobacter*, 2nd edn ed. Nachamkin, I. and Blaser, M.J. pp. 441–454. Washington, DC: American Society for Microbiology.

USDA (2000) *Part III: Health Management and Biosecurity in U.S. Feedlots, 1999*. USDA:APHIS:VS, CEAH, National Animal Health Monitoring System. Fort Collins, CO (no. N336.1200).

Vacher, S., Ménard, A., Bernard, E. and Mégraud, F. (2003) PCR-restriction fragment length polymorphism analysis for detection of point mutations associated with macrolide resistance in *Campylobacter* spp. *Antimicrob Agents Chemother* **47**, 1125–1128.

Van Looveren, M., Daube, G., De Zutter, L., Dumont, J.M., Lammens, C., Wijdooghe, M., Vandamme, P., Jouret, M. *et al.* (2001) Antimicrobial susceptibilities of *Campylobacter* strains isolated from food animals in Belgium. *J Antimicrob Chemother* **48**, 235–240.

Wang, Y. and Taylor, D.E. (1990) Chloramphenicol resistance in *Campylobacter coli*: nucleotide sequence, expression, and cloning vector construction. *Gene* **94**, 23–28.

Wang, Y., Huang, W.M. and Taylor, D.E. (1993) Cloning and nucleotide sequence of the *Campylobacter jejuni gyrA* gene and characterization of quinolone resistance mutations. *Antimicrob Agents Chemother* **37**, 457–463.

Wesley, I.V., Wells, S.J., Harmon, K.M., Green, A., Schroeder-Tucker, L., Glover, M. and Siddique, I. (2000) Fecal shedding of *Campylobacter* and *Arcobacter* spp. in dairy cattle. *Appl Environ Microbiol* **66**, 1994–2000.

White, D.G., Hudson, C., Maurer, J.J., Ayers, S., Zhao, S., Lee, M.D., Bolton, L., Foley, T. *et al.* (2000) Characterization of chloramphenicol and florfenicol resistance in *Escherichia coli* associated with bovine diarrhea. *J Clin Microbiol* **38**, 4593–4598.

