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Contribution of the Multidrug Efflux Transporter CmeABC to Antibiotic Resistance in Different *Campylobacter* Species

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Abstract

CmeABC, a multidrug efflux system in *Campylobacter jejuni*, plays an important role in the resistance to different antimicrobials and toxic compounds. Although this efflux system has been well characterized in *C. jejuni* and to a less extent in *C. coli*, it is unknown if CmeABC homologs are functional in other *Campylobacter* spp. In this study, the *cmeABC* homologs were identified and functionally characterized in five *Campylobacter* species including *C. jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, and *C. fetus*. Our results indicated that *cmeABC* is present in all five *Campylobacter* spp. and the genomic organization of this efflux operon is similar among the *Campylobacter* spp. Insertional mutagenesis of *cmeB* increased the susceptibilities of all the five *Campylobacter* spp. to structurally diverse antimicrobials. Together, these results indicated that the CmeABC efflux system is conserved at both the genomic and functional levels in all five *Campylobacter* spp. examined in this study, further highlighting the significant role of CmeABC in *Campylobacter* pathobiology.

Introduction

Multidrug efflux transporters have been recognized to play a major role in mechanisms responsible for intrinsic and acquired antibiotic resistances in many bacterial species (Poole, 2005). They are membrane transport proteins belonging to several superfamilies, including the ATP binding cassette superfamily, the resistance, nodulation, and cell division (RND) superfamily, the major facilitator superfamily, the small multidrug resistance family, and the multidrug and toxic compound extrusion family (Guillaume *et al.*, 2004; Poole, 2005). The RND-type efflux pump is a tripartite efflux system consisting of an inner membrane transporter, a periplasmic membrane fusion protein, and an outer membrane channel protein (Poole, 2005). RND efflux systems extrude structurally diverse antimicrobials and toxic compounds directly out of bacterial cells (Piddock, 2006).

Campylobacter jejuni is a leading bacterial cause of human enteritis in developed countries (Allos, 2001). Other *Campylobacter* spp., such as *C. coli*, *C. upsaliensis*, *C. lari*, and *C. fetus*, have also been linked to gastrointestinal infections or other clinical diseases (Bourke *et al.*, 1998; Martinot *et al.*, 2001). Gastroenteritis caused by *Campylobacter* is usually self-limiting and does not require antibiotic therapy, but antibiotic treatment is needed in severe and prolonged cases, such as those occurring in immunoincompetent patients (Tee and Mijch, 1998). When clinical therapy is warranted, fluoroquinolone and macrolide antibiotics are the drugs often prescribed for the treatment of *Campylobacter* infections. Development of resistance to these antibiotics in *Campylobacter* reduces the effectiveness of antibiotic therapy and has emerged as a major public health problem worldwide (Luangtongkum *et al.*, 2009).

Multiple mechanisms associated with antibiotic resistance have been identified in *Campylobacter*, but target mutations and drug efflux are most relevant to the resistance to fluoroquinolones and macrolides (Luangtongkum *et al.*, 2009). These two mechanisms function together in conferring high-level resistance to the two classes of antibiotics. In *C. jejuni* an RND-type efflux pump, named CmeABC, was found to mediate the extrusion of structurally diverse antimicrobials and toxic compounds and contributes to the intrinsic and acquired resistances to various antimicrobials (Lin *et al.*, 2002; Pumbwe and Piddock, 2002; Luo *et al.*, 2003). CmeABC is also a major player in the efflux of bile acids and plays a critical role in facilitating *Campylobacter* colonization of the intestinal tract

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Table 1. Bacterial Strains Used in This Study

Name	*Description*	*Source or reference*
Escherichia coli *stains and plasmid*		
JM109	Cloning strain for blue and white colony screening	Promega
pGEM-T easy	Polymerase chain reaction cloning vector, amp^r	Promega
Campylobacter *strains*		
700819	*C. jejuni* strain NCTC 11168	ATCC
S3b	*C. jejuni*, isolated from chicken	This laboratory
993868	*C. coli*, isolated from human	UI[a]
33559	*C. coli*, isolated from swine	ATCC
TF1-13	*C. coli*, isolated from turkey	This laboratory
H-8	*C. coli*, isolated from swine	This laboratory
2744	*C. upsaliensis*	NADC[b]
3121	*C. lari*, isolated from chicken	NADC
3125	*C. lari*, a environmental isolate from sediments	NADC
5652	*C. fetus*, isolated from bovine	NADC
6953	*C. fetus*, isolated from equine	NADC
S3b-B	S3b derivative; *cmeB*::*cat*	This study
99B	993868 derivative; *cmeB*::*cat*	This study
TF99B	TF1-13 derivative; *cmeB*:*cat*	This study
44B	2744 derivative; *cmeB*::*cat*	This study
21B	3121 derivative; *cmeB*::*cat*	This study
25B	3125 derivative; *cmeB*::*cat*	This study
52B	5652 derivative; *cmeB*::*kan*	This study
53B	6953 derivative; *cmeB*::*kan*	This study

[a]From N. Moyer, University of Iowa.
[b]From I.V. Wesley, National Animal Disease Center, U.S. Department of Agriculture.
ATCC, American type culture collection.

(Lin *et al.*, 2003). Expression of *cmeABC* is subject to regulation by CmeR, a repressor encoded by a gene immediately upstream of *cmeA* (Lin *et al.*, 2005a). CmeR binds directly to an inverted repeat in the promoter region of *cmeABC* and inhibits the transcription of this efflux operon. Bile salts induce the expression of *cmeABC*, by inhibiting the binding of CmeR to the promoter of *cmeABC* (Lin *et al.*, 2005a, 2005b).

As discussed earlier, the genetic features and functions of CmeABC have been well defined in *C. jejuni* and to a less extent in *C. coli* (Cagliero *et al.*, 2005). However, it is unknown if this efflux pump is also functional in the same fashion in other *Campylobacter* spp. The purposes of this study were to identify the orthologs of CmeABC and to characterize their function in different *Campylobacter* spp., including *C. jejuni*, *C. coli*, *C. upsaliensis*, *C. lari*, and *C. fetus*. We focused on these five species because they have been linked to clinical diseases in humans (Lastovica and Allos, 2008).

Materials and Methods

Bacterial strains and cultures

The bacterial strains of the five *Campylobacter* spp. used in this study are listed in Table 1. *Campylobacter* strains were grown in Mueller–Hinton (MH) broth or on MH agar plates at either 37°C (for *C. upsaliensis*, *C. lari*, and *C. fetus*) or 42°C (for *C. jejuni* and *C. coli*) under microaerobic conditions. For the culture of *C. upsaliensis*, *C. lari*, and *C. fetus*, the MH broth and agar plates were supplemented with either *Campylobacter* growth supplement (SR0232; Oxoid, Cambridge, UK) or 5% horse blood (Cleveland Scientific, Bath, OH). For the selection and culture of the *cmeB* insertional mutants in different *Campylobacter* spp., either 4 μg/mL of chloramphenicol or 20 μg/mL of kanamycin was added to the corresponding culture media depending on the antibiotic resistance cassette inserted in the mutants. *Escherichia coli* JM109 was routinely grown in Luria–Bertani (LB) medium. For the selection of *E. coli* transformants, the LB medium was supplemented with a final concentration of 30 μg/mL kanamycin or 20 μg/mL chloramphenicol, and 100 μg/mL ampicillin according to the selection marker(s) carried on the plasmid.

DNA extraction and plasmid purification

Campylobacter genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI), following the manufacturer's instructions. *E. coli* plasmids were extracted from overnight cultures in LB broth supplemented with appropriate antibiotics using the Plasmid MiniPrep Kit (Qiagen, Valencia, CA), following the manufacturer's instructions.

cmeABC *sequencing strategies*

Polymerase chain reaction (PCR)-based sequencing in conjunction with direct sequencing of genomic DNA was used in determining the sequences of *cmeABC* in various *Campylobacter* spp. *cmeABC*-specific primers were designed from the genome sequences of *C. jejuni* 11168 and 81–176 for sequencing the *cmeABC* operon in *C. jejuni* and *cmeB* in *C. coli*. For the initial sequencing of the *cmeABC* operon in *C. upsaliensis*, *C. lari*, and *C. fetus*, for which the *cmeABC* DNA could not be amplified using primers designed from *C. jejuni*, a pair of degenerate primers (Table 2) for a fragment of the *cmeB* gene were designed and the amplified DNA was cloned into

Table 2. Key Polymerase Chain Reaction Primers Used in This Study

		Target amplified
Degenerate primers for cmeB of C. upsaliensis, C. lari, *and* C. fetus		
cmeB1213F	GGDATHGTYGTAGATGATGC	*cmeB*
cmeB2817R	GCRAAYTCWACRATYAARATHGCATT	*cmeB*
Mutagenesis primers		
chlU	ACGGATCCAAAGAGTGACCGCCGAGA (BamHI)[a]	*cat* cassette
chlL	ACTCTAGACAGTGCGACAAACTGGGA (XbaI)	*cat* cassette
kanU	ATGGATCCTGCAAGGAACAGTGAAT (BamHI)	*kan*^{r} cassette
kanL	ATTCTAGAGCGATGAAGTGCGTAAG (XbaI)	*kan*^{r} cassette
99UBF	CAATCCGTTAGTACAATGAG	*C. coli cmeB*
99UBR	ACGGATCCCATATTATCTGCGCCATTGA (BamHI)	*C. coli cmeB*
99LBF	GGTCTAGAGGAGTGGATCAGCTTATCAA (XbaI)	*C. coli cmeB*
99LBR	GTATTCTCCTACTGCTCCTA	*C. coli cmeB*
44BUF	CAGCTTGTAAGCGATTATGA	*C. upsaliensis cmeB*
44BUR	ACGGATCCAGCGATAACTTCTAGTATGC (BamHI)	*C. upsaliensis cmeB*
44BLF	ACTCTAGAAGCTAGTATGGCTCTAATCG (XbaI)	*C. upsaliensis cmeB*
44BLR	CTAAGGTAGAAGCAGCTATC	*C. upsaliensis cmeB*
LarUF	TCTAGCTTGTGCTTCGCTTA	*C. lari cmeB*
LarUR	TTGGATCCGAAGAGCTTGAGGTTGAATC (BamHI)	*C. lari cmeB*
LarLF	AATCTAGAGGCCATTGGTATTGTGGTTG (XbaI)	*C. lari cmeB*
LarLR	GTGCAGGATCACCTTGAACT	*C. lari cmeB*
FetUF	TATGCCAGTAACTGTTATGC	*C. fetus cmeB*
FetUR	ACGGATCCTAGTATCGGCTATAGTCTGT (BamHI)	*C. fetus cmeB*
FetLF	ACTCTAGATGTTCGCACTTATACTAGCC (XbaI)	*C. fetus cmeB*
FetLR	TGATACTACCGAGTCTAACG	*C. fetus cmeB*

[a]Restriction sites are underlined and labeled in parentheses.

pGEM-T plasmid and subsequently sequenced. The known *cmeB* sequences of *C. coli*, *C. upsaliensis*, *C. lari*, and *C. fetus* were used to design outward primers, which were then used to obtain the complete *cmeABC* sequences in these *Campylobacter* spp. through direct sequencing of their genomic DNA. DNA sequencing was performed at the Iowa State University DNA Facilities.

Polymerase chain reaction

PCR was performed in a reaction volume of 50 μL containing 200 nM each of the deoxynucleoside triphosphates, 2.0 mM of $MgCl_2$, 200 nM each of the primers, 50–100 ng of template DNA, and 2.5 units of the *Taq* polymerase (Promega) or the *Pfu* Turbo polymerase (Stratagene, La Jolla, CA). PCR cycling conditions were determined according to the estimated annealing temperatures of the primers and the length of the amplified products. PCR products for DNA sequencing and cloning were purified using a commercial PCR purification kit (Qiagen).

Mutagenesis of the CmeABC efflux pump

The *cmeB* mutant was generated for each *Campylobacter* spp. through the insertion of a chloramphenicol (*cat*) or kanamycin (*kan*^{r}) resistance cassette in the *cmeB* gene based on the previously published method with minor modifications (Akiba *et al.*, 2006). PCR primers used for the mutagenesis are listed in Table 2. In the first step of mutagenesis, a suicide vector carrying either a *cat* or *kan*^{r} cassette flanked by 0.5–1.2 kb of the *cmeB* fragments was constructed. Briefly, the *cat* or *kan*^{r} cassette was amplified using the *Pfu* polymerase (Stratagene) from pUOA18 (*cat*) or pMW10 plasmids (*kan*^{r}) and purified with a PCR purification kit (Qiagen). The antibiotic cassette was inserted into the *cmeB* sequence by direct ligation or an inverse PCR method. For the direct ligation method, the amplified *cmeB* fragments and the antibiotic cassette were treated with restriction enzymes and then ligated by T4 DNA ligase (Promega). The ligated DNA with *cmeB* DNA flanking the antibiotic cassette was purified by agarose gel electrophoresis. The DNA band of the expected size was excised and ligated to pGEM-T (Promega) through A–T cloning. For the inverse PCR method, the *cmeB* DNA was amplified by the *Taq* polymerase and cloned into pGEM-T, which was then transformed into *E. coli* JM109 cells. The purified pGEM-T constructs were used as templates in the inverse PCR using the *Pfu* polymerase and a pair of *cmeB* inverse PCR primers. The amplified DNA was ligated to the antibiotic cassette by T4 DNA ligase, resulting in a suicide vector carrying the antibiotic cassette flanked by the *cmeB* sequences. The suicide vectors were transformed into *Campylobacter* cells via electroporation according to the protocol described previously (Guerry *et al.*, 1994). After overnight incubation on a nonselective MH agar plate, the electroporated cells were plated onto selective MH agar plate with appropriate antibiotics and incubated for 3–5 days microaerobically at 42°C. Single colonies of transformants were obtained and the *cmeB* insertional mutations were confirmed by PCR.

Minimal inhibitory concentration tests on Campylobacter *strains and mutants*

The broth microdilution method was used to measure the minimal inhibitory concentration (MIC) of various antimicrobials as described previously (Lin *et al.*, 2002). *C. jejuni* 11168 was used as an in-house quality control. Fresh *Campylobacter* cultures were grown for 24–48 hours in MH (for

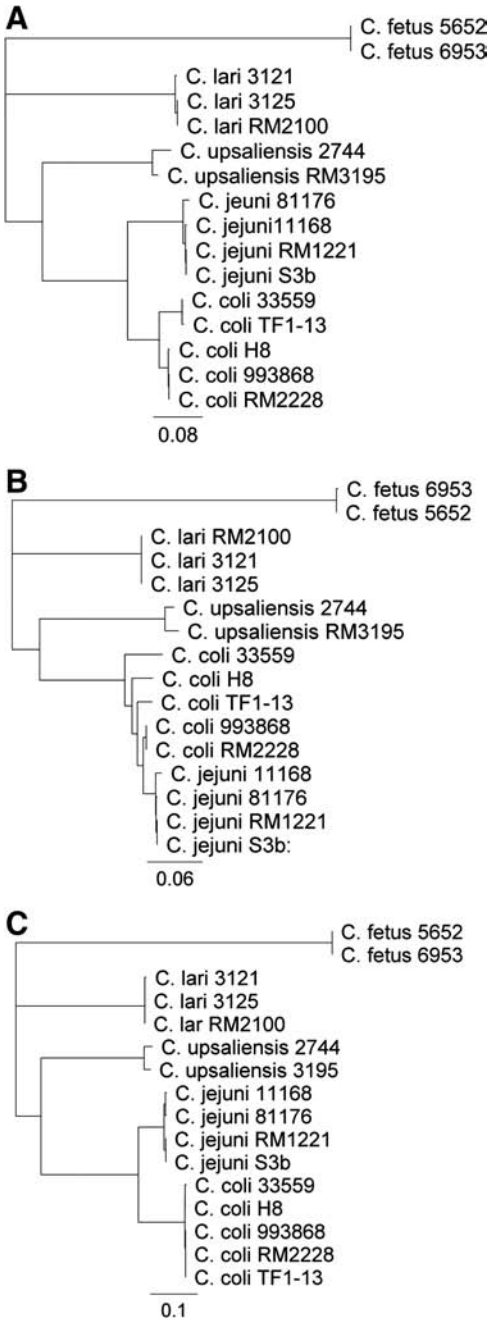
C. jejuni and *C. coli*) or MH with *Campylobacter* growth supplement (for *C. upsaliensis*, *C. lari*, *and C. fetus)* and used as inocula. The supplement (SR0232; Oxoid) contains no antibiotics, but sodium pyruvate, sodium metabisulphite, and ferrous sulphate, and was used to stimulate the growth of the slow-growing *Campylobacter* spp. Twofold dilution series of antibiotics or chemicals in 50 μL of the same bacterial culture media were made in 96-well plates. Fifty microliters of *Campylobacter* culture containing 5×10^5 colony forming unit (CFU) cells/mL were added to the 50 μL of diluted antibiotics or chemicals in the 96-well plates and mixed well by gentle shaking before incubation. The plates were incubated at 37°C (*C. upsaliensis*, *C. lari*, and *C. fetus*) or 42°C (*C. jejuni* and *C. coli*) for 48 hours under microaerobic conditions. The MIC test was repeated at least three times.

Sequence alignment

The obtained DNA sequences were assembled and analyzed by Vector NIT software (Invitrogen, Carlsbad, CA). The CmeABC sequences were aligned by the ClustalW software and phylogenetic trees were constructed by the neighbor-joining method.

Sequence accession number

The GenBank accession numbers of the CmeABC sequences used in this study are FJ797669, FJ797670, FJ797671, FJ797672, FJ797673, FJ797674, FJ797675, FJ797676, FJ797677, and FJ797678.



Results and Discussion

Comparison of the cmeABC *operon in different* Campylobacter *spp.*

Five species of *Campylobacter* were examined in this study, including *C. jejuni*, *C. coli*, *C. upsaliensis*, *C. lari*, and *C. fetus*. The genomic organization of the CmeABC operon was conserved among all examined *Campylobacter* spp., with one nucleotide overlapping between *cmeA* and *cmeB* and eight nucleotides overlapping between *cmeB* and *cmeC*. The *cmeR* gene, which encodes a local repressor of CmeABC in *C. jejuni* (Lin *et al.*, 2005a), was also identified in all of the *Campylobacter* spp. and it is located immediately upstream of *cmeABC*. *Cj0364*, encoding a hypothetical protein with an unknown function, is immediately downstream of *cmeABC* in *C. jejuni* and this is also the case in *C. coli* and *C. upsaliensis*. These findings are consistent with the results of a previous study conducted in *C. coli* (Corcoran *et al.*, 2005). However, in *C. lari* and *C. fetus*, *cmeABC* is followed by an open reading frame that is distinct from *Cj0364* (data not shown).

Sequence analysis showed that *cmeB* and *cmeC* are the most and least conserved, respectively, in the operon among the five *Campylobacter* species. Based on the amino acid sequences of CmeABC, the phylogenetic relatedness of the *Campylobacter* species in relation to *C. jejuni* was in the order of *C. coli*, *C. upsaliensis*, *C. lari*, and *C. fetus* (Fig. 1), which is in agreement with the finding from a previous report by comparing 12 conserved protein sequences (Fouts *et al.*, 2005), but different from the phylogenetic tree constructed from the 16S rRNA sequences (Vandamme, 2000). As the number of strains of each species sequenced in this study was limited, we also included the CmeABC sequences available in GenBank for alignment, which showed that within each *Campylobacter* species, the

FIG. 1. Dendrograms constructed with the amino acid sequences of CmeA (**A**), CmeB (**B**), and CmeC (**C**) from different *Campylobacter* species. The rooted trees were constructed with the neighbor-joining method based on ClustalW alignments of the CmeA, CmeB, and CmeC amino sequences of *Camplylobacter* strains from this study (*C. jejuni* S3b; *C. coli* 993868, 33559, TF1-13, and H-8; *C. upsaliensis* 2744; *C. lari* 3121and 3125; *C. fetus* 5652 and 6953) and representative genome sequences deposited in GenBank (*C. jejuni* 11168, 81176, and RM1221; *C. coli* RM2228; *C. upsaliensis* RM3195; and *C. lari* RM2100).

TABLE 3. MINIMAL INHIBITORY CONCENTRATIONS (μG/ML) OF VARIOUS ANTIMICROBIALS IN DIFFERENT *CAMPYLOBACTER* SPP. AND THEIR ISOGENIC *cmeB* MUTANTS

Antimicrobials	C. jejuni *11168*	C. jejuni *S3B*	C. coli *993868*	C. coli *TF1-13*	C. upsaliensis *2744*	C. lari *3121*	C. fetus *5652*
Ciprofloxacin	0.25/0.031 **(8)**	0.5/0.063 **(8)**	0.25/0.031 **(8)**	0.25/0.031 **(8)**	0.5/0.063 **(8)**	32/2 **(16)**	1/0.0625 **(16)**
Erythromycin	1/0.063 **(16)**	1/0.031 **(32)**	1/0.031 **(32)**	256/4 **(64)**	0.125/0.087 **(16)**	2/0.031 **(64)**	1/0.25 **(4)**
Ampicillin	8/2 **(4)**	16/1 **(16)**	256/128 **(2)**	16/8 **(2)**	1/0.25 **(4)**	2/0.125 **(16)**	8/0.25 **(4)**
Cephalothin	256/8 **(32)**	256/4 **(64)**	2048/32 **(64)**	2048/8 **(256)**	2/0.25 **(8)**	128/0.5 **(256)**	16/0.0625 **(256)**
Cefoperazone	256/16 **(16)**	1024/16 **(64)**	2048/128 **(16)**	1024/32 **(32)**	16/0.063 **(256)**	256/0.125 **(2048)**	256/0.125 **(2048)**
Cefotaxime	8/0.063 **(128)**	16/0.063 **(256)**	16/0.25 **(64)**	16/0.063 **(256)**	1/0.063 **(16)**	32/0.063 **(512)**	8/0.016 **(512)**
Rifampin	256/1 **(256)**	256/0.5 **(512)**	64/0.0625 **(1024)**	128/0.25 **(512)**	8/0.063 **(128)**	64/0.5 **(128)**	128/128 **(1)**
Gentamicin	2/1 **(2)**	2/1 **(2)**	1/0.5 **(2)**	1/0.5 **(2)**	8/8 **(1)**	16/16 **(1)**	32/32 **(1)**
Tetracycline	0.125/0.063 **(2)**	64/8 **(8)**	0.5/0.125 **(4)**	128/8 **(16)**	1/0.125 **(8)**	8/0.5 **(16)**	8/0.5 **(16)**
Polymyxin B	8/4 **(2)**	4/2 **(2)**	2/4 **(0.5)**	2/4 **(0.5)**	2/4 **(0.5)**	4/8 **(0.5)**	>512/>512 (–)
Ethidium bromide	1/0.125 **(8)**	0.5/0.0625 **(8)**	8/0.5 **(16)**	4/0.25 **(16)**	8/0.25 **(32)**	4/1 **(4)**	16/1 **(16)**
Cholic acid	8192/128 **(64)**	8192/128 **(64)**	4096/256 **(16)**	8192/256 **(32)**	2048/32 **(64)**	8192/32 **(256)**	8192/128 **(64)**
Sodium dodecyl sulphate	16/4 **(4)**	32/4 **(8)**	16/4 **(4)**	32/8 **(4)**	64/8 **(8)**	256/8 **(32)**	256/16 **(16)**

Values indicate minimal inhibitory concentrations of the wild-type/its isogenic *cmeB* mutant. Values in bold are fold changes.

CmeABC sequences are highly conserved, with amino acid identities ranging from 95% to 100% (data not shown). This finding is consistent with a previous report in which the alignment of partial *cmeC* sequences from 131 *C. jejuni* and *C. coli* strains indicated the conserved nature of CmeC sequences within each species (Fakhr and Logue, 2007). However, another study reported a variant CmeB in *C. jejuni* that was only 80% identical to that of *C. jejuni* 81–176 (Cagliero *et al.*, 2006).

The *cmeR* sequence is also conserved among the different *Campylobacter* spp. The inverted repeat, which serves as a binding site for CmeR (Lin *et al.*, 2005a), was also identified in the intergenic region between *cmeR* and *cmeA* for all *Campylobacter* spp. (data not shown). These findings suggest that the CmeABC efflux system and its regulator CmeR are conserved in *Campylobacter* spp. and this system is important for *Campylobacter* pathobiology.

CmeABC contributes to antibiotic resistance in different Campylobacter spp.

In this study, the *cmeB* homologs in different *Campylobacter* spp. were inactivated successfully by insertional mutagenesis. The mutants and their corresponding wild-type strains were compared for susceptibility to various antibiotics and the results are listed in Table 3. The mutagenesis of CmeB resulted in a substantial increase in the susceptibility of all *Campylobacter* spp. to the majority of antibiotics and toxic compounds examined in this study.

There were 8- to 16- and 4- to 64-fold changes in the MICs of ciprofloxacin and erythromycin, respectively (Table 3). It was known from previous studies that CmeABC and target mutations, such as the 23S rRNA and *gyrA* mutations, work synergistically to confer high-level resistance to macrolide and fluoroquinolone antibiotics in *C. jejuni* and *C. coli* (Luo *et al.*, 2003; Cagliero *et al.*, 2005; Mamelli *et al.*, 2005). The high-level resistance of *C. coli* TF1-13 to erythromycin and *C. lari* 3121 to ciprofloxacin observed in this study was likely owing to the presence of resistance-conferring mutations in these two isolates. Regardless, mutation of *cmeB* in these two isolates resulted in a significant reduction in the MICs of ciprofloxacin or erythromycin (Table 3), further indicating the importance of CmeABC in conferring resistance to the antibiotics. The *cmeB* mutation also led to 2- to 16-fold reduction in the MICs of tetracycline. The resistance to gentamicin was slightly affected by the *cmeB* mutation in *C. jejuni* and *C. coli* (twofold change in the MIC), but not in *C. upsaliensis*, *C. lari*, and *C. fetus*. The *cmeB* mutation also affected the susceptibility to ampicillin in different *Campylobacter* spp., with 2- to16-fold changes in the MIC observed.

All *Campylobacter* spp. examined in this study were highly resistant to cephalothin and cefoperazone and moderately resistant to cefotaxime (Table 3). Consistent and drastic decreases in MICs of cephalosporin antibiotics (8- to 2048-fold) were observed in the *cmeB* mutants of different *Campylobacter* species. Previously, the high-level intrinsic resistance of *Campylobacter* to cephalosporins was attributed to the low binding affinity of the antibiotics to the bacterial penicillin-binding protein or the low permeability of the cell membrane (Lachance *et al.*, 1991). The results from this study suggest that the efflux mediated by CmeABC also plays a significant role in the resistance to these cephalosporin antibiotics in different *Campylobacter* spp.

Campylobacter is intrinsically resistant to rifampin, and mutation of CmeABC was shown to cause a dramatic decrease in the MIC of rifampin in *C. jejuni*. Similar results were observed in this study with *C. coli*, *C. upsaliensis*, and *C. lari*; however, no changes in the MIC of rifampin occurred in the *cmeB* mutant of *C. fetus* (Table 3). This finding suggests that CmeABC is important for the intrinsic resistance to rifampin in *C. jejuni*, *C. coli*, *C. upsaliensis*, and *C. lari*, but does not play a role in rifampin resistance in *C. fetus*. Rifampin resistance in other bacteria is conferred by mutations in the DNA-dependent RNA polymerase (RpoB) (Musser, 1995), but in some *Campylobacter* spp. the resistance appears to be mainly mediated by efflux. Because the *cmeB* mutation did not affect the MIC of rifampin in *C. fetus*, the resistance in this species is likely due to a different mechanism.

The *cmeB* mutation also significantly increased the susceptibility to cholic acid in all five species of *Campylobacter*, indicating that this efflux pump plays a major role in the adaptation to bile-containing environments such as the intestinal tract (Table 3). The resistance to sodium dodecyl sulphate and ethidium bromide was also decreased (Table 3). These results are consistent with previous findings with CmeABC in *C. jejuni* and indicate that CmeABC is important for the resistance to antimicrobial compounds in different *Campylobacter* spp.

Several antibiotics, such as rifampin, cephalothin, and cefoperazone, have been incorporated into commercial *Campylobacter* selective media for the isolation of *Campylobacter* spp. from clinical samples (Karmali *et al.*, 1981; Goossens *et al.*, 1986; Ng *et al.*, 1988; Burnens and Nicolet, 1992). The results in this study indicate that CmeABC is associated with high-level intrinsic resistance to these antibiotics and our data also provide a molecular explanation for the use of these antibiotics as *Campylobacter* selective agents. On the other hand, the results from this study also revealed that *C. upsaliensis* appears to be intrinsically more susceptible to the often used selective antibiotics, which highlights the need to lower the concentration of antibiotics in selective media to successfully recover *C. upsaliensis* from clinical samples. It has been reported that the use of filtration has a significantly better recovery rate of *C. upsaliensis* in clinical samples than using selective medium (Lastovica and Le Roux, 2001). Further work in understanding the antimicrobial resistance mechanisms will facilitate the selection of antibiotics for clinical treatment of campylobacteriosis and the formulation of diagnostic media for various *Campylobacter* spp.

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Disclosure Statement

No competing financial interests exist.

References

Akiba M, Lin J, Barton YW, and Zhang Q. Interaction of CmeABC and CmeDEF in conferring antimicrobial resistance and maintaining cell viability in *Campylobacter jejuni*. J Antimicrob Chemother 2006;57:52–60.

Allos BM. *Campylobacter jejuni* infections: update on emerging issues and trends. Clin Infect Dis 2001;32:1201–1206.

Bourke B, Chan VL, and Sherman P. *Campylobacter upsaliensis*: waiting in the wings. Clin Microbiol Rev 1998;11:440–449.

Burnens AP and Nicolet J. Detection of *Campylobacter upsaliensis* in diarrheic dogs and cats, using a selective medium with cefoperazone. Am J Vet Res 1992;53:48–51.

Cagliero C, Cloix L, Cloeckaert A, and Payot S. High genetic variation in the multidrug transporter *cmeB* gene in *Campylobacter jejuni* and *Campylobacter coli*. J Antimicrob Chemother 2006;58:168–172.

Cagliero C, Mouline C, Payot S, and Cloeckaert A. Involvement of the CmeABC efflux pump in the macrolide resistance of *Campylobacter coli*. J Antimicrob Chemother 2005;56:948–950.

Corcoran D, Quinn T, Cotter L, O'Halloran F, and Fanning S. Characterization of a *cmeABC* operon in a quinolone-resistant *Campylobacter coli* isolate of Irish origin. Microb Drug Resist 2005;11:303–308.

Fakhr MK and Logue CM. Sequence variation in the outer membrane protein-encoding gene *cmeC*, conferring multidrug resistance among *Campylobacter jejuni* and *Campylobacter coli* strains isolated from different hosts. J Clin Microbiol 2007;45:3381–3383.

Fouts DE, Mongodin EF, Mandrell RE, Miller WG, Rasko DA, Ravel J, Brinkac LM, DeBoy RT, Parker CT, Daugherty SC, Dodson RJ, Durkin AS, Madupu R, Sullivan SA, Shetty JU, Ayodeji MA, Shvartsbeyn A, Schatz MC, Badger JH, Fraser CM, and Nelson KE. Major structural differences and novel potential virulence mechanisms from the genomes of multiple *Campylobacter* species. PLoS Biol 2005;3:e15.

Goossens H, De Boeck M, Coignau H, Vlaes L, Van den Borre C, and Butzler JP. Modified selective medium for isolation of *Campylobacter* spp. from feces: comparison with Preston medium, a blood-free medium, and a filtration system. J Clin Microbiol 1986;24:840–843.

Guerry P, Yao R, Alm RA, Burr DH, and Trust TJ. Systems of experimental genetics for *Campylobacter* species. Methods Enzymol 1994;235:474–481.

Guillaume G, Ledent V, Moens W, and Collard JM. Phylogeny of efflux-mediated tetracycline resistance genes and related proteins revisited. Microb Drug Resist 2004;10:11–26.

Karmali MA, De Grandis S, and Fleming PC. Antimicrobial susceptibility of *Campylobacter jejuni* with special reference to resistance patterns of Canadian isolates. Antimicrob Agents Chemother 1981;19:593–597.

Lachance N, Gaudreau C, Lamothe F, and Lariviere LA. Role of the beta-lactamase of *Campylobacter jejuni* in resistance to beta-lactam agents. Antimicrob Agents Chemother 1991;35:813–818.

Lastovica AJ and Allos BM. Clinical significance of *Campylobacter* and related species other than *Campylobacter jejuni* and *Campylobacter coli*. In: *Campylobacter*, 3rd edition. Nachamkin I, Szymanski CM, and Blaser MJ (eds.). Washington, DC: ASM Press, 2008, pp. 123–149.

Lastovica AJ and Le Roux E. Efficient isolation of *Campylobacter upsaliensis* from stools. J Clin Microbiol 2001;39:4222–4223.

Lin J, Akiba M, Sahin O, and Zhang Q. CmeR functions as a transcriptional repressor for the multidrug efflux pump CmeABC in *Campylobacter jejuni*. Antimicrob Agents Chemother 2005a;49:1067–1075.

Lin J, Cagliero C, Guo B, Barton YW, Maurel MC, Payot S, and Zhang Q. Bile salts modulate expression of the CmeABC

multidrug efflux pump in *Campylobacter jejuni*. J Bacteriol 2005b;187:7417–7424.

Lin J, Michel LO, and Zhang Q. CmeABC functions as a multidrug efflux system in *Campylobacter jejuni*. Antimicrob Agents Chemother 2002;46:2124–2131.

Lin J, Sahin O, Michel LO, and Zhang Q. Critical role of multidrug efflux pump CmeABC in bile resistance and *in vivo* colonization of *Campylobacter jejuni.* Infect Immun 2003;71: 4250–4259.

Luangtongkum T, Jeon B, Han J, Plummer P, Logue CM, and Zhang Q. Antibiotic resistance in *Campylobacter*: emergence, transmission, and persistence. Future Microbiol 2009;4:189–200.

Luo N, Sahin O, Lin J, Michel LO, and Zhang Q. *In vivo* selection of *Campylobacter* isolates with high levels of fluoroquinolone resistance associated with *gyrA* mutations and the function of the CmeABC efflux pump. Antimicrob Agents Chemother 2003;47:390–394.

Mamelli L, Prouzet-Mauleon V, Pages JM, Megraud F, and Bolla JM. Molecular basis of macrolide resistance in *Campylobacter*: role of efflux pumps and target mutations. J Antimicrob Chemother 2005;56:491–497.

Martinot M, Jaulhac B, Moog R, De Martino S, Kehrli P, Monteil H, and Piemont Y. *Campylobacter lari* bacteremia. Clin Microbiol Infect 2001;7:96–97.

Musser JM. Antimicrobial agent resistance in mycobacteria: molecular genetic insights. Clin Microbiol Rev 1995;8: 496–514.

Ng LK, Taylor DE, and Stiles ME. Characterization of freshly isolated *Campylobacter coli* strains and suitability of selective media for their growth. J Clin Microbiol 1988;26:518–523.

Piddock LJ. Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. Clin Microbiol Rev 2006;19:382–402.

Poole K. Efflux-mediated antimicrobial resistance. J Antimicrob Chemother 2005;56:20–51.

Pumbwe L and Piddock LJ. Identification and molecular characterisation of CmeB, a *Campylobacter jejuni* multidrug efflux pump. FEMS Microbiol Lett 2002;206:185–189.

Tee W and Mijch A. *Campylobacter jejuni* bacteremia in human immunodeficiency virus (HIV)-infected and non-HIV-infected patients: comparison of clinical features and review. Clin Infect Dis 1998;26:91–96.

Vandamme P. Taxonomy of the family Campylobacteraceae. In: *Campylobacter*, 2nd edition. Nachamkin I and Blaser MJ (eds.). Washington, DC: ASM Press, 2000, pp. 3–26.

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