Journal of Antimicrobial Chemotherapy (2009) **63**, 462–468
doi:10.1093/jac/dkn529
Advance Access publication 15 January 2009

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Roles of lipooligosaccharide and capsular polysaccharide in antimicrobial resistance and natural transformation of *Campylobacter jejuni*

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Received 2 September 2008; returned 16 October 2008; revised 20 November 2008; accepted 12 December 2008

***Objectives*: This study is aimed to determine the role of capsular polysaccharide (CPS) and lipooligosaccharide (LOS) in modulating antimicrobial resistance and natural transformation of *Campylobacter jejuni*, an important food-borne human pathogen.**

***Methods*: A series of *C. jejuni* mutants, which are defective in either CPS or LOS or both, were constructed. The antimicrobial susceptibility, bacterial surface hydrophobicity, natural transformation frequency and DNA binding and uptake were measured and compared between the mutants and the wild-type strain.**

***Results*: Truncation of LOS greatly reduced (8-fold) the intrinsic resistance of *C. jejuni* to erythromycin, a key antibiotic used for treating human campylobacteriosis, while the loss of CPS did not result in significant changes in the susceptibility to antimicrobial agents. Notably, mutation of LOS also significantly increased (>16-fold) the susceptibility to erythromycin in *C. jejuni* mutants carrying the A2074G mutation in 23S rRNA. The increased susceptibility to erythromycin in the LOS mutant was probably due to enhanced permeability to this antibiotic, because the LOS mutation rendered the surface of *C. jejuni* more hydrophobic. Loss of CPS and truncation of LOS increased the transformation frequency by 4- and 25-fold, respectively, and mutation of both CPS and LOS resulted in a 97-fold increase in the transformation frequency. Consistent with the increased transformation frequencies, the CPS and LOS mutants showed enhanced rates of DNA uptake.**

***Conclusions*: These results demonstrate that the surface polysaccharides in *C. jejuni* contribute to the resistance to erythromycin, a clinically important antibiotic, but restrict natural transformation.**

Keywords: food safety, macrolides, competence, drug susceptibility

Introduction

Gram-negative bacteria possess surface polysaccharides such as capsular polysaccharide (CPS) and lipopolysaccharide (LPS) on the outer membrane. These surface polysaccharides are attached to bacterial cells by covalent linkages to their lipid moieties.[1,2] Since the surface polysaccharides constitute the outermost layer of the Gram-negative bacterial cell, they directly interact with the surrounding environments, providing a permeability barrier to noxious antimicrobial agents and conferring resistance to host immunity.[1,3] The LPS structure embedded in the outer leaflet of the outer membrane considerably decreases the permeability of hydrophobic compounds through the outer membrane and contributes to the general resistance of Gram-negative bacteria to hydrophobic antibiotics such as macrolides.[1,4]

Campylobacter jejuni is a Gram-negative bacterium and a major food-borne pathogen frequently associated with human gastroenteritis worldwide.[5] More than 2 million cases of *Campylobacter* infections occur in the USA each year.[6] *Campylobacter* infection is characterized by diarrhoea, fever and abdominal cramps and occasionally causes Guillain–Barré syndrome as a post-infection complication.[7] When clinical treatments are warranted, macrolides and fluoroquinolones are the drugs of choice to treat *Campylobacter* infections.[7] However, the resistance

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Table 1. PCR primers used in this study

Primers	Sequence (5′ to 3′)
kpsS_F	GCT CAA GTT GAA GAT GAT GCT TCG ATG AT
kpsS_R	CAT ACC AAA ACA GGA TTG GGT TTA TAA GCA TGA
Kan_F	CTTATCAATATATCCATGGAATGGGCAAAGCAT
Kan_R	GATAGAACCATGGATAATGCTAAGACAATCACTAAA
waaF_F	CCA AAC CGA CCA GCA AAA ATG CCT TTG T
waaF_R	GAT GAA GAC ACG CCT TTA GAA CTT ATA AGC TT
TetOF	TTA TTT TTG CAT AAA CAG ATG ATT AGT G
TetOR	GCA AGC TGT TAA GCT AAC TTG T
waaF_comF	CCC AAA TTT CTA CTT GCA AAA GTG CCC AAA
waaF_comR	GGT ATT TCA ACG AGC GGA AAA AGC CCT AAT

of *Campylobacter* to both classes of antibiotics has been on the rise and is considered a threat to public health.[8] Several general mechanisms involved in bacterial resistance to antibiotics have been described, including antibiotic inactivation, target modification, active efflux and reduced cell permeability.[9] The first three general mechanisms have been well studied in *Campylobacter*,[10] but the influence of cell surface structures on the susceptibility to clinically important antibiotics by altering cell permeability is still poorly understood in this bacterium.

One of the unique features of *C. jejuni* is its ability to take up DNA by natural transformation.[11] Our recent work showed that natural transformation plays an important role in horizontal transfer of antibiotic resistance determinants in *Campylobacter*.[12] As a first step during the process of natural transformation in Gram-negative bacteria including *C. jejuni*, free DNA in the environment must interact with the surface polysaccharide layer prior to contacting the competence proteins in the cell membrane. Thus, it is conceivable that alteration of the surface polysaccharide structures would affect the efficiency of natural transformation.

C. jejuni possesses both CPS and lipooligosaccharide (LOS).[13] The LOS differs from LPS in that LOS does not possess an O-antigen polysaccharide chain.[13] These surface polysaccharide structures are highly variable among different *Campylobacter* strains[14,15] and are related to the virulence of this bacterium.[16,17] Molecular mimicry between *C. jejuni* LOS and human gangliosides is also implicated in Guillain–Barré syndrome, a paralytic neuropathy.[13] Despite the advances in understanding the structures and functions of CPS and LOS, their roles in the natural transformation and antibiotic resistance in *Campylobacter* remain to be determined. In a previous study, Kanipes *et al.*[16] demonstrated that *C. jejuni* LOS is associated with resistance to fusidic acid, novobiocin and SDS, but its contribution to clinically important antibiotics, such as fluoroquinolones and macrolides, was not determined in that work. In this study, we demonstrated that LOS, but not CPS, plays an important role in *Campylobacter* resistance to erythromycin and that surface polysaccharides restrict the natural transformation frequency of *C. jejuni* NCTC 11168.

Materials and methods

Bacterial strains and culturing conditions

C. jejuni NCTC 11168 and its derivatives were used in this study. *C. jejuni* strains JL271, JL272 and JL273 are highly resistant to erythromycin (MIC >512 mg/L), carry the A2074G mutation in 23S rRNA and were derived from chickens fed with tylosin in a previous study.[18] JB304 is a derivative of strain 11168, which was constructed in this study by transformation with the genomic DNA of strain JL271 and is highly resistant to erythromycin. The strains were grown in Mueller–Hinton (MH) medium under microaerobic (5% O_2/10% CO_2/85% N_2) conditions at 42°C. When needed, the culture medium was supplemented with kanamycin (50 mg/L), tetracycline (5 mg/L) or chloramphenicol (10 mg/L).

Construction of CPS and LOS mutants

To construct a CPS mutant, a 1693 bp region harbouring *kpsS* was PCR-amplified with kpsS_F and kpsS_R primers (Table 1) and cloned into pGEM-T (Promega, Madison, WI, USA) to yield pGEM-T::*kpsS*. The pGEM-T::*kpsS* plasmid was digested with *Swa*I and ligated with the *aphA3* gene, which was amplified from pMW10[19] with Kan_F and Kan_R (Table 1). The suicide vector was introduced to *C. jejuni* 11168 by electroporation, and the resulting mutants were selected on MH agar plates containing kanamycin (50 mg/L). The mutation in *kpsS* was confirmed by PCR (data not shown) and the mutant was named JB301. For constructing an LOS mutant, a 2206 bp region containing *waaF* was amplified with *Ex Taq*™ (TaKaRa Bio Inc., Japan) using primers waaF_F and waaF_R (Table 1) and cloned into pGEM-T to yield pGEM-T::*waaF*. Digestion of pGEM-T::*waaF* with *Bgl*II removed a 649 bp internal fragment of *waaF*, and the construct was blunt-ended by treatment with Klenow fragment (TaKaRa Bio Inc.). The tetracycline resistance cassette (*tetO*) was amplified with Vent$_R^®$ DNA polymerase (New England Biolab, Ipswich, MA, USA) from pTet in *C. jejuni* 81-176,[20] using TetOF and TetOR primers (Table 1). The *tetO* gene was inserted into the *waaF* on pGEM-T::*waaF*, and the construct was electroporated into *C. jejuni* 11168. The resulting transformants were selected by growth on MH agar plates containing tetracycline (5 mg/L). The *waaF* mutant was confirmed by PCR (data not shown) and was named JB302. The double mutant JB303, which carries mutations in both *kpsS* and *waaF*, was constructed by transforming JB301 with the genomic DNA of JB302. The double mutant was selected by growth on MH agar plates containing both tetracycline (5 mg/L) and kanamycin (50 mg/L).

waaF and *kpsS* mutants were also constructed with macrolide-resistant *C. jejuni* strains (JB304, JL271, JL272 and JL273). The *kpsS* and *waaF* mutants of these strains were constructed by transforming these strains using the genomic DNA of JB301 (*kpsS*::*aphA3*) and JB302 (*waaF*::*tetO*), respectively, and the

transformants were selected by growth on MH agar plates containing kanamycin (50 mg/L) or tetracycline (5 mg/L). The insertional mutations in *kpsS* and *waaF* of these erythromycin-resistant strains were confirmed by PCR (data not shown).

Detection of LOS and CPS by Alcian Blue staining

CPS and LOS preparation and Alcian Blue staining were performed according to the previously reported method.[21] Briefly, a loopful of 1-day-old *C. jejuni* culture grown on MH agar plates was resuspended in lysis buffer containing 62.5 mM Tris–HCl, pH 6.8, 2% SDS, 10% glycerol and 0.05% Bromophenol Blue. Samples were boiled for 10 min and centrifuged at 13000 rpm for 5 min. Aliquots of the supernatant were taken and mixed with proteinase K (Sigma, St Louis, MO, USA) to a final concentration of 1 mg/mL. The samples were incubated at 50ºC for 1 h and then fractionated by SDS–PAGE. LOS and CPS were visualized with Alcian Blue staining (0.1% Alcian Blue dissolved in 40% ethanol/5% acetic acid).

Complementation of the waaF mutant in trans

Construction of the complementing plasmid for the *waaF* mutant was based on a previously published study.[16] A 1450 bp region containing *waaF* and its upstream intergenic region was amplified with $\text{Vent}_{\text{R}}^{®}$ DNA polymerase, using waaF_comF and waaF_comR primers (Table 1). The PCR product was cloned into the *Sma*I site of pRY112. The constructed plasmid was introduced into the *waaF* mutant of *C. jejuni* strains 11168 (JB302), JL271 and JL273 by conjugation, as described previously.[22] The *waaF*-complemented strain JB302 was named JB302C.

Susceptibility tests

The MICs of various antibiotics, SDS and choleate were determined with a microtitre broth dilution method as described previously.[23] Antimicrobial agents were purchased from Sigma (erythromycin, cefotaxime, gentamicin, rifampicin, polymyxin B, streptomycin, SDS and choleate) and MP Biomedicals, Irvine, CA, USA (ciprofloxacin).

Hydrophobicity assay

Hydrophobicity of *C. jejuni* strains was measured by a salting-out method as described by Misawa and Blaser.[24] Briefly, overnight cultures of *C. jejuni* strains were resuspended in 2 mM sodium phosphate (Sigma) to an optical density at 600 nm (OD_{600}) of 1.0. Ammonium sulphate (4 M) was dissolved in 2 mM sodium phosphate and 2-fold serially diluted in U-bottomed 96-well plates. An equal volume (25 μL) of bacterial suspension was added to each well containing ammonium sulphate. The plates were incubated at room temperature overnight. The minimum concentration of ammonium sulphate forming bacterial aggregation was determined and used for the hydrophobicity index. Hydrophobicity is inversely correlated with the concentration of ammonium sulphate causing bacterial aggregation.

Natural transformation

Natural transformation was performed as described previously[11] with some modifications. *C. jejuni* cultures grown overnight on MH agar plates were collected and resuspended in MH broth to OD_{600} of 0.05. Bacterial suspensions (0.5 mL) were transferred to sterilized tubes, incubated at 37°C with shaking (200 rpm) under microaerobic conditions for 3 h and then 1 μg of genomic DNA of JB201 or JB202,[12] which carry the chloramphenicol resistance gene (*cat*) and the kanamycin resistance gene (*aphA3*), respectively, was added to each tube. After additional incubation for 4 h, bacterial cultures were serially diluted and plated on MH agar plates with or without antibiotics (10 mg/L chloramphenicol or 50 mg/L kanamycin) to count the numbers of transformants and the total bacterial number, respectively. Transformation frequency was calculated by dividing the number of transformants from 1 μg of donor DNA by the total number of bacteria.

DNA binding and uptake assay

Genomic DNA from *C. jejuni* NCTC 11168 was extracted using the Wizard Genomic DNA Purification Kit (Promega). Up to 400 ng of chromosomal DNA was nick translated using the Nick Translation Kit (Roche, Indianapolis, IN, USA) in the presence of 100 μCi of [α-^{32}P]dCTP (Perkin Elmer, Waltham, MA, USA) as recommended by the manufacturer. Unincorporated nucleotides were removed by ethanol precipitation and the DNA was resuspended in 82 μL of double distilled water. The CPS and LOS mutants and the wild-type 11168, grown for 18 h on MH agar, were resuspended in 10 mL MH broth to an OD_{600} of 0.1. The culture tubes were incubated microaerobically with shaking (250 rpm) until the optical density had doubled (~3 h). Cells were harvested by centrifugation at 4000 **g** for 20 min and resuspended in 3.3 mL MH broth. Each culture was adjusted to the same optical density at OD_{600} to ensure an equivalent number of cells were used in the DNA binding and uptake assay for each strain. To each culture (1 mL), 100 ng of ^{32}P-labelled DNA was added, and the culture was incubated microaerobically with labelled DNA for 2 h and divided into two 500 μL aliquots. DNaseI was added to the aliquots for DNA uptake assay to a final concentration of 100 mg/L to digest extracellular DNA. After DNase I treatment for 10 min at room temperature, cells were washed three times with MH broth and resuspended in 100 μL of ice-cold water prior to liquid scintillation (Beckman Coulter). Since the intracellular DNA is protected from DNaseI treatment, the amount of radioactivity from the samples treated with DNaseI represents the level of DNA uptake. The radioactivity of the sample without DNaseI treatment was considered the DNA-binding level. Three independent experiments were performed.

Statistical analysis

Normality and variance of each dataset were assessed by D'Agostino Omnibus[25] and Modified-Levene test,[26] respectively. Differences between the wild-type and each mutant strain in hydrophobicity were tested using the Mann–Whitney *U*-test, and the Student *t*-test was used for the transformation frequency dataset. For the analysis of the results of DNA binding and uptake, ANOVA was used with the strain and DNaseI treatment as fixed variables, and the Bonferroni corrected multiple comparison *t*-test was applied to variables with significant main or interaction effects.

Results

Contribution of LOS, but not CPS, to the intrinsic resistance to erythromycin

Multiple genes clustered in two separate loci are involved in LOS and CPS biosynthesis in *C. jejuni*. Based on their importance in LOS and CPS production,[27,28] we selected *waaF* and *kpsS* as

Table 2. Antimicrobial susceptibility of *C. jejuni* 11168 and its derivatives

	MIC (mg/L)[a]				
Antimicrobials	11168	JB301	JB302	JB303	JB302C
Ciprofloxacin	0.5	0.5	0.5	0.5	0.5
Erythromycin	0.5	0.5	0.0625 (8)	0.0313 (16)	0.25
Cefotaxime	1	2 (0.5)	1	0.5 (2)	1
Gentamicin	1	2 (0.5)	1	1	1
Rifampicin	>128	>128	>128	>128	>128
Polymyxin B	1	2 (0.5)	0.5 (2)	0.5 (2)	1
Streptomycin	0.5	1 (0.5)	0.5	0.5	0.5
SDS	128	64 (2)	64 (2)	32 (4)	128
Choleate	32 768	32 768	32 768	32 768	32 768

[a]The numbers in parentheses indicate the differences (*n*-fold) between wild-type 11168 and the mutant strains. JB301, CPS mutant; JB302, LOS mutant; JB303, CPS and LOS double mutant; JB302C, JB302 carrying pRY112::*waaF*.

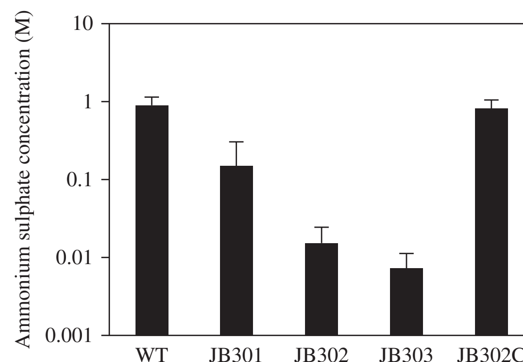

Figure 1. Effect of CPS and LOS mutation on surface hydrophobicity of *C. jejuni*. The concentration of ammonium sulphate is inversely proportional to hydrophobicity. Each bar represents the mean ± SEM of three different experiments. WT, *C. jejuni* 11168; JB301, CPS mutant (*kpsS*::*aphA3*); JB302, LOS mutant (*waaF*::*tetO*); JB303, CPS and LOS double mutant; JB302C, JB302 harbouring pRY112::*waaF*.

target genes to disrupt LOS and CPS, respectively. The *waaF* gene encodes a heptosyltransferase, while *kpsS* encodes a putative CPS transporter. Truncation of LOS and loss of CPS in these mutants were confirmed by Alcian Blue staining [Figure S1, available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/)]. The LOS-complemented strain (JB302C) partially restored the production of intact LOS (Figure S1a). Thus, both the truncated form and the intact form of LOS were seen in JB302C. The growth rate of the CPS and LOS mutants in MH broth was comparable to that of the wild-type (data not shown).

The antimicrobial susceptibilities of the CPS and LOS mutants were compared with that of the wild-type. As shown in Table 2, the mutations had different effects on the sensitivity to various antibiotics. Although JB301 (the CPS mutant) and JB302 (the LOS mutant) did not exhibit any changes in the susceptibility to ciprofloxacin (a hydrophilic fluoroquinolone), JB302 showed a significant increase (8-fold) in the susceptibility to erythromycin. JB302C (JB302 complemented with a plasmid-carried copy of *waaF*) had MIC levels similar to those of the wild-type (Table 2). The LOS mutation also moderately (2-fold) increased the susceptibility of *Campylobacter* to polymyxin B and SDS. These changes were reproducible in four independent experiments. In contrast to LOS, the mutation of CPS did not result in an increase in the susceptibilities of the tested antimicrobials except a minor change (2-fold) in SDS. Instead, the CPS mutant showed slightly decreased sensitivity to some hydrophilic antibiotics, such as cefotaxime, gentamicin, polymyxin B and streptomycin. Compared with JB302, the MICs of erythromycin, cefotaxime and SDS were further reduced in JB303 (a CPS and LOS double mutant), but the reduction was modest (Table 2), suggesting that the MIC changes in the double mutant were mainly due to the LOS mutation.

Effect of the LOS mutation on the susceptibility to erythromycin in macrolide-resistant mutants

High-level macrolide resistance in *Campylobacter* is often associated with point mutations in the 23S rRNA.[29] To investigate if LOS affects the resistance to macrolide antibiotics in *C. jejuni* strains harbouring the A2074G mutation in 23S rRNA, we introduced the LOS and CPS mutations to JB304, JL271, JL272 and JL273, which are highly resistant to erythromycin (MIC >512 mg/L). While the CPS mutation did not have a measurable effect on macrolide resistance in these *C. jejuni* strains, the LOS mutation decreased the MIC of erythromycin from >512 to 16–32 mg/L (a >16-fold reduction). *In trans* complementation of the LOS mutants of JL271 and JL273 restored the high-level resistance to erythromycin (MIC >512 mg/L).

Increased hydrophobicity in CPS and LOS mutants

We hypothesized that the loss of hydrophilic surface polysaccharides in the CPS and LOS mutants would make the bacterial surface more hydrophobic than that of the wild-type. To test this hypothesis, we measured the hydrophobicity of the strains by the salting-out method. In this assay, a higher concentration of ammonium sulphate mediating bacterial aggregation indicates lower hydrophobicity.[24] Thus, the concentration of ammonium sulphate is inversely proportional to hydrophobicity. CPS mutation was previously reported to increase the hydrophobicity of *C. jejuni*.[30] In our study, we confirmed the previous finding regarding the *C. jejuni* CPS mutant and further demonstrated LOS had a much greater impact on surface hydrophobicity than CPS. Mutation of CPS and LOS increased the hydrophobicity by 3.2- and 93-fold, respectively, as reflected by the changes in the concentration of ammonium sulphate (Figure 1). The CPS and LOS double mutation resulted in a 171-fold increase in the hydrophobicity (Figure 1). The differences between the wild-type and each mutant were statistically significant ($P<0.001$) by the Mann–Whitney *U*-test. These results demonstrated that CPS and LOS are strongly associated with the surface hydrophobicity of *C. jejuni*.

Contribution of CPS and LOS to natural transformation

We performed natural transformation with these mutants and found that the CPS and LOS mutations increased the frequency

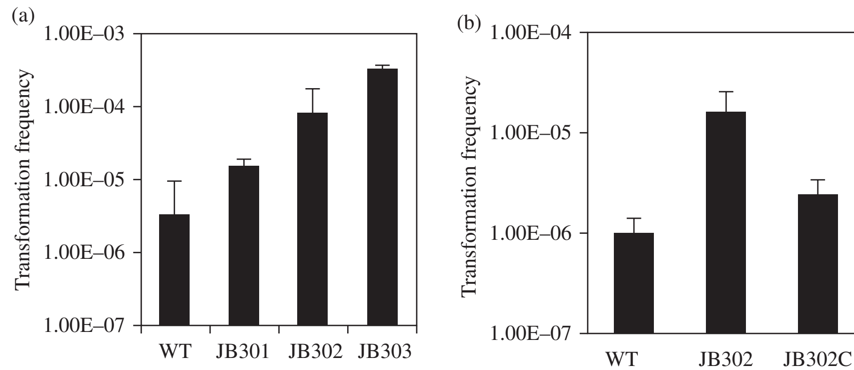

Figure 2. Effect of CPS and LOS mutation on natural transformation in *C. jejuni*. (a) Transformation frequencies in various mutant constructs. The result is representative of four independent experiments with triplicate transformation reactions. The donor DNA contained a chloramphenicol resistance marker (*cat*). Each bar represents the mean ± SEM. WT, *C. jejuni* 11168; JB301, CPS mutant; JB302, LOS mutant; JB303, CPS and LOS double mutant. (b) Complementation of the *waaF* mutant in natural transformation. JB302C is JB302 complemented with pRY112::*waaF*. The result is representative of three independent experiments with triplicate transformation. Since the complementing plasmid itself carried a *cat* gene, the genomic DNA containing the kanamycin resistance marker (*aphA3*) was used as the donor DNA. Each bar represents the mean ± SEM.

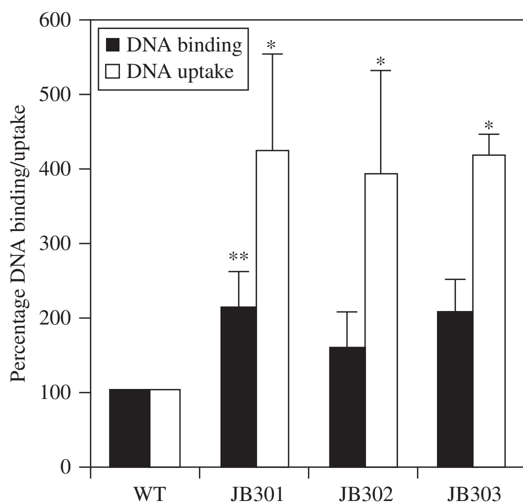

Figure 3. Effect of CPS and LOS mutation on DNA binding and uptake in *C. jejuni*. The result is representative of three independent experiments. Each bar represents the mean ± SEM of three samples in one experiment. In the repeated experiments, similar patterns of results were obtained (see the Results section). The statistically significant difference between each mutant and the wild-type strain is indicated by one asterisk ($P<0.01$) or two asterisks ($P<0.05$). WT, *C. jejuni* 11168; JB301, CPS mutant; JB302, LOS mutant; JB303, CPS and LOS double mutant.

of natural transformation by 4- and 25-fold, respectively, and the double mutation of both LOS and CPS significantly increased the transformation frequency by 97-fold (Figure 2a). The results were reproducible in four independent experiments. The difference between the wild-type and each mutant was statistically significant ($P<0.001$). Since a greater change in transformation was observed with the LOS mutant than with the CPS mutant, we complemented the LOS mutant using a wild-type *waaF* carried on a shuttle plasmid. *In trans* complementation decreased the transformation frequency to a near wild-type level (Figure 2b). The differences between the wild-type and the *waaF*-complemented strain were statistically insignificant ($P>0.1$).

CPS and LOS affected DNA binding and uptake

DNA binding and uptake assays were performed to determine whether the LOS and CPS mutations affect DNA binding and uptake in *Campylobacter*. The results demonstrated that DNA binding and uptake increased 2- and 4-fold, respectively, in the mutants (Figure 3). Despite variations observed between experiments in the measured radioactivity levels, the fold differences between the wild-type and the mutant strains remained consistent. The fold changes in DNA uptake were statistically significant in all of the mutants ($P<0.01$). The increase in DNA binding was statistically significant ($P<0.05$) in JB301, but was marginally non-significant in JB302 and JB303 as determined by Bonferroni corrected *t*-test despite the trend of increased DNA binding (Figure 3). Although JB303 consistently showed a higher transformation frequency than JB301 and JB302 (Figure 2a), it did not demonstrate greater DNA binding and uptake rates than the single mutants (Figure 3).

Discussion

The role of surface polysaccharides in mediating antibiotic resistance in *C. jejuni* was determined in this study. Although a previous study reported that the LOS mutation sensitized *C. jejuni* to fusidic acid, novobiocin and SDS,[16] it was unknown if CPS also contributes to antimicrobial resistance and if both CPS and LOS are important for resisting clinically relevant antibiotics, such as fluoroquinolones and macrolides. In this study, we showed that *C. jejuni* LOS plays a significant role in the protection against the action of erythromycin (Table 2), which is hydrophobic and a key antibiotic clinically used to treat *Campylobacter* infection. Compared with LOS, CPS had a limited impact on antimicrobial resistance in *C. jejuni* (Table 2). Instead, the CPS mutation in *C. jejuni* slightly decreased the susceptibility to some hydrophilic antibiotics, such as cefotaxime, gentamicin, streptomycin and polymyxin B (Table 2).

Campylobacter resistance to erythromycin is associated with mutations in 23S rRNA[29] and the function of the multidrug efflux pump CmeABC.[18] In this study, we showed that truncation of LOS reduced *Campylobacter* resistance to erythromycin

(Table 2). In other bacteria, it has been shown that loss of LPS renders bacterial cells more permeable to hydrophobic agents and consequently increases the sensitivity to hydrophobic antibiotics.[1,4] Many Gram-negative bacteria such as *E. coli* and *Salmonella* Typhimurium are intrinsically resistant to hydrophobic antimicrobial agents including erythromycin, and this intrinsic resistance to erythromycin is ascribed to the exclusion of hydrophobic antibiotics by the hydrophilic LPS.[1,4] Since *C. jejuni* only has LOS and does not have the full-length LPS, it is much more susceptible to macrolides than *E. coli* and *Salmonella*, explaining why macrolide antibiotics are suitable for treating *Campylobacter* infections. In this study, we showed that truncation of LOS further increased the susceptibility of *Campylobacter* to erythromycin. Unlike hydrophilic antibiotics, which traverse the outer membrane through water-filled channels such as porins, hydrophobic macrolides diffuse through the outer membrane via hydrophobic interaction.[1,4] Based on the fact that truncation of LOS significantly increased the hydrophobicity of *Campylobacter* (Figure 1), we speculate that the LOS mutant was more permeable to erythromycin and thus more susceptible to the antibiotic than the wild-type strain.

Although both CPS and LOS affected the hydrophobicity of *C. jejuni* (Figure 1), loss of CPS alone did not alter the bacterial susceptibility to erythromycin (Table 2). This was probably due to the possibility that LOS alone provides a sufficient barrier for diffusion of macrolides across the outer membrane in *Campylobacter*. WaaF is responsible for the addition of the second heptose to the inner-core region of *C. jejuni* LOS, and its mutation results in the formation of truncated LOS[16,28] (Figure S1a). The LOS mutant containing the *waaF* mutation showed a slight (2-fold) reduction in the MIC level of polymyxin B (Table 2). The increased sensitivity to SDS by the LOS mutation was reported in a previous study[16] and was confirmed by our result (Table 2). However, the CPS and LOS mutants did not show any changes in the susceptibility to choleate (Table 2), indicating that the surface polysaccharides do not contribute significantly to bile resistance.

Natural transformation is an important mechanism of horizontal gene transfer and significantly mediates the spread of antimicrobial resistance determinants in *Campylobacter*.[12,31] Natural transformation requires many protein components and is distinct from other artificial DNA transformations such as heat-shock transformation and electroporation.[32] Although the role of LPS in conjugation and heat-shock transformation was reported in naturally non-competent *Salmonella* Typhimurium,[33,34] to our knowledge this is the first report documenting the role of bacterial polysaccharides in natural transformation. In this study, we showed that bacterial surface polysaccharides, especially LOS, reduced the transformation frequencies in *C. jejuni* (Figure 2). How the surface polysaccharides reduce natural transformation in *Campylobacter* may be explained by two possibilities. First, both DNA and the surface polysaccharides are negatively charged and electrostatic repulsion between them may restrict the contact of transforming DNA with the bacterial surface. Second, CPS and LOS may act as physical barriers, sterically and/or structurally hindering the binding of DNA to receptor protein(s) located in the outer membrane. In either case, the surface polysaccharide would reduce the rate of DNA uptake by bacterial cells. Indeed, our results showed that loss of CPS and LOS significantly increased DNA uptake into *Campylobacter* cells (Figure 3), supporting the notion that surface polysaccharides limit the interaction of transforming DNA with specialized DNA binding and uptake apparatuses in the membrane. JB303 (double mutant) showed a higher transformation frequency than the single mutants (Figure 2a), but the measured rate of DNA binding and uptake in JB303 was not higher than those of the single mutants (Figure 3). The reason for this discrepancy is unknown, but it might be explained by the low sensitivity of the DNA uptake assay, which did not allow the differentiation of DNA uptake rates among the mutants.

It was previously reported that the *galE* mutation in *C. jejuni* strain 81116 resulted in the formation of truncated LOS and a 20-fold decrease in the natural transformation frequency, suggesting that the LOS mutation reduced the natural transformation frequency of this *C. jejuni* strain,[35] which is in contrast to the findings obtained in this study. However, GalE is an epimerase with dual functions that converts glucose to galactose and *N*-acetylglucosamine to *N*-acetylgalactosamine[36] and is required for three important pathways including CPS biosynthesis, LOS production and N-linked protein glycosylation.[13,37] Thus, mutation of *galE* would result in pleiotropic changes and affect the production of LOS and CPS[35,36] as well as the N-linked protein glycosylation.[36] Since the N-linked protein glycosylation plays a significant role in the natural transformation of *C. jejuni*,[38] it is likely that the reduced natural transformation frequency in the *C. jejuni galE* mutant was not caused by the truncation of LOS, but was due to an altered N-linked protein glycosylation system.

In conclusion, we demonstrated in this study that loss of LOS, but not CPS, significantly reduced *Campylobacter* resistance to erythromycin, an important antibiotic for treating human campylobacteriosis. It was also shown that loss of LOS and CPS increased the frequency of natural transformation in *C. jejuni* strain NCTC 11168, which may ultimately affect the horizontal transfer of antibiotic resistance determinants. Given that both CPS and LOS are highly diverse and phase variable in *Campylobacter*,[13,30] the results from this study suggest that the variable production of CPS and LOS in different *Campylobacter* strains may contribute to their intrinsic differences in antimicrobial susceptibility and natural transformation of antibiotic resistance genes.

Acknowledgements

We would like to thank Jun Lin at the University of Tennessee for providing some of the erythromycin-resistant isolates used in this study.

Funding

This study was supported by the National Institutes of Health grant R01DK063008 and the National Research Initiative competitive grant 2007-35201-18278 from the USDA Cooperative State Research, Education, and Extension Service.

Transparency declarations

None to declare.

Supplementary data

Figure S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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