Journal of Food Protection, Vol. 69, No. 4, 2006, Pages 768–774

Adherence to and Invasion of Human Intestinal Epithelial Cells by *Campylobacter jejuni* and *Campylobacter coli* Isolates from Retail Meat Products

JIE ZHENG,[1] JIANGHONG MENG,[1] SHAOHUA ZHAO,[2] RUBY SINGH,[2] AND WENXIA SONG[3]*

[1]*Department of Nutrition & Food Science and* [3]*Department of Cell Biology & Molecular Genetics, University of Maryland, College Park, Maryland 20742; and* [2]*Center for Veterinary Medicine, Office of Research, U.S. Food and Drug Administration, Laurel, Maryland 20708, USA*

MS 05-273: Received 31 May 2005/Accepted 5 September 2005

ABSTRACT

The abilities of 34 *Campylobacter jejuni* and 9 *Campylobacter coli* isolates recovered from retail meats to adhere to and invade human intestinal epithelial T84 cells were examined and compared with those of a well-characterized human clinical strain, *C. jejuni* 81-176, to better assess the pathogenic potential of these meat isolates. The meat isolates exhibited a wide range of adherence and invasion abilities; a few of the isolates adhered to and invaded T84 cells almost as well as did *C. jejuni* 81-176. There was a significant correlation between the adherence ability and the invasion ability of the *Campylobacter* isolates. The presence of eight putative virulence genes in these *Campylobacter* isolates that are potentially responsible for adherence and invasion or that encode cytolethal distending toxin was determined using PCR. All *Campylobacter* isolates possessed *flaA, cadF, pldA, cdtA, cdtB,* and *cdtC,* and most (91%) also contained the *ciaB* gene. However, the *virB11* gene, carried by virulence plasmid pVir, was absent in almost all the *Campylobacter* isolates. Our findings indicated that *C. jejuni* and *C. coli* present in retail meat were diverse in their ability to adhere to and invade human intestinal epithelial cells and that the putative virulence genes were widespread among the *Campylobacter* isolates. Thus, despite of the presence of the putative virulence genes, only some but not all *Campylobacter* strains isolated from retail meat can effectively invade human intestinal epithelial cells in vitro.

Campylobacter is one of the leading causes of acute bacterial diarrhea worldwide *(32).* Numerous surveys have revealed retail meat and raw milk as sources of human infection *(2).* A high percentage of poultry products is contaminated with *Campylobacter (14, 36, 51).*

Campylobacter organisms cause disease by at least three mechanisms: (i) intestinal colonization by ingested organisms and production of bacterial cytotoxin *(8, 16, 20),* inducing diarrhea, (ii) bacterial invasion of intestinal cells *(44, 50),* resulting in damage to mucosal surface cells of jejunum, ileum, and colon, and (iii) extraintestinal translocation *(15, 27),* in which the organisms cross the intestinal epithelium and migrate via the lymphatic system to various extraintestinal sites. These possible mechanisms are not mutually exclusive, and *Campylobacter* may make use of any combination of the mechanisms depending on the host status and attributes of the infection strain. The molecular basis of pathogenicity of *Campylobacter* has not been fully elucidated. However, several virulence factors have been identified based on in vitro *(10, 12, 25, 43)* and in vivo *(6, 46, 52)* studies. For example, *flaA,* which encodes flagellin *(47),* and *cadF,* which encodes a protein that interacts with a host extracellular matrix protein fibronectin *(35),* are required for *Campylobacter* adherence to and colonization of the host cell surface. Other genes such as *ciaB (26, 42), pldA (42),* and genes of the pVir plasmid *(3)* are involved in host cell invasion. The genes *cdtA, cdtB,* and *cdtC (29, 41)* are responsible for the expression of *Campylobacter* cytolethal distending toxin, which induces the proinflammatory cytokine production of epithelial cells *(18)* and causes host cell cycle arrest, cell distention, and eventually cell death *(48).*

The ability of *Campylobacter jejuni* to adhere to and invade the epithelial cells of the gastrointestinal tract is important for the development of *Campylobacter*-mediated enteritis *(40, 46).* The adherence to and invasion of *C. jejuni* into host cells has been studied in a variety of cell lines *(11, 19, 23, 39).* Human colonic epithelial cell line T84 has been widely used to assess the ability of enteric bacteria to adhere to and invade the epithelium. *C. jejuni* strain 81-176, a well-studied human clinical strain *(28),* has a relatively high invasion efficiency in several human intestinal epithelial cell lines, including T84 *(19, 39),* and causes illness in human volunteers *(6).*

Despite frequent contamination of retail meat with *Campylobacter,* it remains unclear whether all *Campylobacter* isolates in retail meat are pathogenic to humans and which factors specifically contribute to their pathogenicity. Methodologies for specifically identifying and distinguishing pathogenic strains of *Campylobacter* from nonpathogenic strains are needed for food safety surveillance. The present study was undertaken to characterize the adherence and invasion abilities of *Campylobacter* isolated from retail meat. The method involved use of the T84 cell culture mod-

* Author for correspondence. Tel: 301-405-7552; Fax: 301-314-9489; E-mail: wenxsong@umd.edu.

el and determination of the prevalence of eight putative virulence genes in these isolates.

MATERIALS AND METHODS

Bacterial strains. Forty-three *Campylobacter* isolates (34 *C. jejuni* and 9 *C. coli*) were selected based on their distinct DNA fingerprinting pulsed-field gel electrophoresis profiles (data not shown) from 378 *Campylobacter* isolates recovered from retail raw meats collected in the Washington, D.C., area from June 1999 to July 2000 *(51).* The 43 isolates were recovered from chicken ($n = 36$), turkey ($n = 4$), pork ($n = 2$), and beef ($n = 1$) samples. The isolates were allowed to grow through two passages before being used for the analyses. A well-characterized human clinical strain, *C. jejuni* 81-176 (kindly provided by Dr. P. Guerry-Kopecko, Naval Medical Research Center, Bethesda, Md.) *(17, 38),* and a noninvasive *Escherichia coli* laboratory strain, DH5α, were used as positive and negative controls for all the analyses, respectively.

Bacterial growth conditions. *Campylobacter* cells were routinely grown on Mueller-Hinton (MH) agar (Difco, Becton Dickinson, Sparks, Md.) containing 5% (vol/vol) citrated sheep blood at 37°C under a microaerophilic atmosphere (85% N_2, 10% CO_2, and 5% O_2). *E. coli* DH5α was cultured aerobically on Luria-Bertani agar (Sigma, St. Louis, Mo.) at 37°C. All bacteria were subcultured for 18 h before being used in experimental assays. Bacteria were harvested from plates with phosphate-buffered saline (PBS; 0.01 M sodium phosphate, pH 7.4, and 0.15 M NaCl) and diluted to a concentration of approximately 10^8 CFU/ml, which was determined using a spectrophotometer (SmartSpec 3000, Bio-Rad, Hercules, Calif.) at a wavelength of 600 nm. Titration and serial dilution analyses revealed that an absorbance of 0.1 corresponded to a *Campylobacter* concentration of 4.09×10^8 CFU/ml.

Cell culture. T84 cells (human colonic epithelial cell line ATCC CCL-248) were cultured in a 1:1 mixture of Dulbecco's modified Eagle's medium (DME) and Ham's F12 medium containing 1.2 g/liter sodium bicarbonate, 2.5 mM L-glutamine, 15 mM HEPES, and 0.5 mM sodium pyruvate (Invitrogen, San Diego, Calif.) supplemented with 5% (vol/vol) fetal bovine serum, penicillin, and streptomycin (5% DME-F12), as recommended by the American Type Culture Collection (Manassas, Va.). Cells were seeded onto 24-well tissue culture plates (Costar, Cambridge, Mass.) at 2×10^5 cells per well and grown for 24 h under 5% CO_2 at 37°C before adherence and invasion assays.

Adherence and invasion assay. The adherence and invasion assays were performed using T84 cells cultured in 24-well tissue culture plates as previously described *(23).* T84 cells were rinsed five times with prewarmed invasion medium (5% DME-F12 without penicillin and streptomycin) and inoculated with 10^6 CFU of bacteria in 1 ml of invasion medium per well, with a multiplicity of infection of about 10 to 20. The number of inoculated bacteria was determined simultaneously on MH–blood agar plates. T84 cells were incubated with bacteria for 3 h in a humidified 5% CO_2 incubator at 37°C to allow bacteria to adhere and invade. The T84 cells were then washed five times with PBS to remove bacteria that had not yet adhered and were lysed with 1% saponin (Sigma) for 5 min to release cell-associated bacteria. Serial dilutions of the lysates were plated on MH–blood agar plates and incubated at 37°C for 48 h, and *Campylobacter* colonies were enumerated to determine the number of cell-associated bacteria.

To determine the number of bacteria that had been internalized, T84 cells that were incubated for 3 h with bacteria were washed three times with PBS and incubated for an additional 2 h with 5% DME-F12 containing 100 μg/ml gentamicin (Sigma) to kill extracellular bacteria. After incubation, cells were washed three times with PBS to remove gentamicin and then lysed with 1% saponin to release intracellular bacteria. The serial dilutions of the lysates were plated on MH–blood agar plates, and the number of CFUs was considered the number of bacteria that had invaded the T84 cells. Each assay was performed in triplicate wells and was repeated at least three times. The number of adhered or internalized bacteria was plotted as a percentage of the starting viable inoculum. Control studies were conducted to verify that a 2-h exposure of the 43 *Campylobacter* isolates to 100 μg/ml gentamicin resulted in 100% kill (data not shown). Time course analyses (0 to 4 h) were carried out to determine the optimal length of time for the incubation of T84 cells with *Campylobacter* (data not shown).

The correlation between the adherence to and invasion of the T84 cells by *Campylobacter* isolates was analyzed by linear regression using SPSS 10.0 software (SPSS Inc., Chicago, Ill.) to calculate Spearman correlation coefficient.

Detection of virulence genes. PCR was used to detect eight *Campylobacter* genes that are associated with virulence in the genomic and plasmid DNA of *Campylobacter* isolates: *flaA, cadF, ciaB, pldA, virB11, cdtA, cdtB,* and *cdtC.* PCR primers specific for these genes were designed based on the gene sequence information in the GenBank database and in previous published studies (Table 1). Conserved sequences of each gene were selected, and two different primer sets were used for each gene. Template DNAs for PCR were extracted by a boiling method as described previously *(33).* Fresh cultures of *Campylobacter* isolates and the negative control strain (*E. coli* DH5α) were suspended in 1 ml of saline and boiled at 100°C for 20 min. After centrifugation at $14{,}000 \times g$ for 2 min, the supernatants were collected and stored at −20°C until use. For detecting *virB11,* both genomic DNA and plasmid DNA from each *Campylobacter* isolate were used as PCR templates. PCR was carried out using Ampli*Taq* Gold polymerase (Roche Molecular Biochemicals, Mannheim, Germany) with 30 cycles of amplification in a GeneAmp PCR system 9600 thermocycler (Perkin-Elmer, Foster City, Calif.). The cycling program was denaturation at 94°C for 1 min, annealing at a temperature specific to each primer pair for 45 s, and extension at 72°C for 45 s. PCR products were separated on 2% agarose gels, and bands were stained with ethidium bromide and visualized using a Gel Doc 1000 imaging system (Bio-Rad).

RESULTS

Adherence and invasion by *Campylobacter* retail meat isolates. To test the pathogenic properties of *Campylobacter* retail meat isolates, the adherence and invasion abilities of 43 *Campylobacter* isolates from retail raw chicken, turkey, beef, and pork were analyzed with T84 human intestinal epithelial cells using a gentamicin resistance assay *(23).* Figure 1A shows the adherence and invasion abilities of each isolate, and Figure 2 displays the distribution of the adherence and invasion abilities of the 43 *Campylobacter* meat isolates tested. After a 3-h incubation, the 43 *Campylobacter* isolates adhered to T84 cells at 0.002 to 3.115% of the starting viable inoculum (Fig. 1A) compared with 1.790% adherence of the positive control strain *C. jejuni* 81-176. Half of the isolates adhered to the cells at 0.002 to 0.170%, i.e., less than one-tenth the adherence of *C. jejuni* 81-176, whereas five isolates (12%) adhered to the cells at 0.590 to 3.115% (Fig. 2), close to

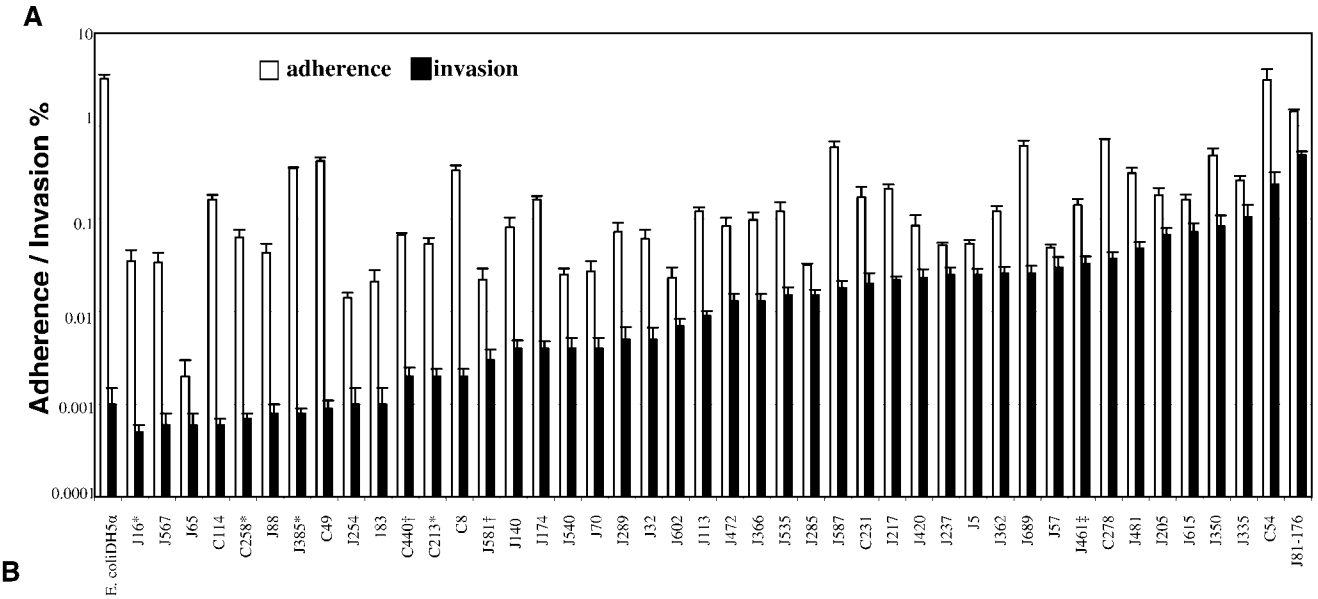
TABLE 1. Campylobacter *virulence genes and primer sequences used for PCR identification*

Target gene	Primers	Sequence (5′ to 3′)	Product (bp)	GenBank accession no.	Reference
C. jejuni cadF	*cadF*-278	ATGGTTTAGCAGGTGGAGGA	252	AF104302, AF104303	This study
	cadF-510	GAGCTGGACGAGTATCAGCA			
C. coli cadF	*cadF*-310	GGTTTAGCTGGTGGGGGATA	525	CJ11168X5, CJU87559	
	cadF-815	CAGAAAGACGGGCTGAAAGC			
cadF	*cadF*-F2B	TTGAAGGTAATTTAGATATG	400		*24*
	cadF-R1B	CTAATACCTAAAGTTGAAAC			
cdtA	*cdtA*-284	TCCTTTGGGGCGTTCATTTG	534	AF038283, U51121	This study
	cdtA-798	ACCGCTGTATTGCTCATAGG			
cdtB	*cdtB*-1094	TCATCCGCAGCCACAGAAAG	203		
	cdtB-1277	ACCCTATCAGGCCTTGAAAG			
cdtC	*cdtC*-1630	TCCAACTAGCGCAACTCAAG	493		
	cdtC-2103	GCTCCAAAGGTTCCATCTTC			
cdt genes	GNW	GGNAAYTGGATHTGGGGNTA			*13*
	WMI1	RTTRAARTCNCCYAADATCATCC			
	VAT2	GTNGCNACBTGGAAYCTNCARGG			
	LPF-D	DAAYTGNACDTANCCDAANGG			
ciaB	*ciaB*-652	TGCGAGATTTTTCGAGAATG	527	AJ312325, AF114831, CJ11168X3	This study
	ciaB-1159	TGCCCGCCTTAGAACTTACA			
	ciaB-403	TTTTTATCAGTCCTTA	986		*9*
	ciaB-1373	TTTCGGTATCATTAGC			
C. jejuni pldA	*pldA*-361	AAGAGTGAGGCGAAATTCCA	385	AJ786391, Y11031	This study
	pldA-726	GCAAGATGGCAGGATTATCA			
C. coli pldA	*pldA*-539	GCTTATAGTTTTGGCTCATTAG	637	AL139078	
	pldA-1154	TACAACAAACGCTTACAAAGAC			
pldA	*pldA*-U	AGATGAATTATTTYYTACCT	600		
	pldA-L	TTGTTRTARTCTATAAGGCT			
flaA	*flaA*-440	TCCAAATCGGCGCAAGTTCA	217	AF345999, AF140252	This study
	flaA-637	TCAGCCAAAGCTCCAAGTCC			
virB11	*virB11*-235	TGTGAGTTGCCTTACCC	395	AF472533	This study
	virB11-612	TTATTTATCGCCGTTTGA			
	virB-232	TCTTGTGAGTTGCCTTACCCCTTTT	494		*9*
	virB-710	CCTGCGTGTCCTGTGTTATTTACCC			

the adherence of *C. jejuni* 81-176. One of the isolates from a chicken meat sample, *C. coli* C54, had the greatest adherent ability among all strains tested. Its adherence (3.115%) was even greater than that of *C. jejuni* 81-176 (1.790%). No significant difference was detected ($P > 0.05$) between *C. jejuni* and *C. coli* strains in their ability to adhere to T84 cells. The nonpathogenic *E. coli* DH5α also had a high level of adherence, similar to that *C. coli* C54, which suggests that high adherence of bacteria to host epithelium does not necessarily lead to infection.

After a 3-h incubation, the percentages of the *Campylobacter* isolates resistant to extracellular gentamicin treatment were 0.001 to 0.235% of the starting viable inoculum (Fig. 1A). The invasion abilities of the 43 raw meat isolates were all lower than that of *C. jejuni* 81-176 (0.315%). Among the 43 meat isolates, 18 isolates (42%) showed invasion levels below 0.004%, similar to that of the noninvasive negative control *E. coli* DH5α (0.001%) but much lower than that of *C. jejuni* 81-176. All of the four turkey isolates belonged to this group. Six raw meat isolates had invasion levels above 0.040%, at about one-tenth of the invasion ability of *C. jejuni* 81-176. The remaining 19 isolates (56%) had invasion levels between 0.004 and 0.040% (Figs. 1A and 2). *C. coli* C54, which was isolated from a chicken sample, had the highest level of invasion (0.235%) among the strains tested, similar to that of *C. jejuni* 81-176 (0.315%). Compared with *C. jejuni*, *C. coli* isolates had a similar range of invasion abilities; however, six of nine *C. coli* isolates tested had invasion levels lower than 0.004%. These results indicate that the abilities of *Campylobacter* meat isolates to adhere to and invade T84 cells vary widely, and only a small percentage of the isolates have adherence and invasion abilities similar to those of the well-characterized human clinical strain *C. jejuni* 81-176.

Correlation between the adherence and invasion abilities of *Campylobacter* retail meat isolates. To test the interrelationship between *Campylobacter* adherence and invasion events, we determined the correlation coefficient between *Campylobacter* adherence and invasion variables using linear regression (Fig. 3). The correlation coefficient (r) between the adherence and invasion efficiency of the *Campylobacter* retail meat isolates was 0.543 ($P < 0.01$), indicating a significantly positive relationship between the invasion and adherence variables. However, there were a few



B

	E. coliDH5α	J16*	J567	J65	C114	C258*	J88	J385*	C49	J254	183	C440†	C213*	C8	J581†	J140	J174	J540	J70	J289	J32	J602	J113	J472	J366	J535	J285	J587	C231	J217	J420	J237	J5	J362	J689	J57	J461‡	C278	J481	J205	J615	J350	J335	C54	J81-176
flaA	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
cadF	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ciaB	-	+	+	+	+	+	+	-	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
pldA	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
virB11	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
cdtA	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
cdtB	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
cdtC	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

FIGURE 1. *The ability of* Campylobacter *retail meat isolates to adhere to and invade T84 human intestinal epithelial cells and the distribution of virulence genes among these isolates. (A) A gentamicin resistance assay (23) was used to test 43* Campylobacter *strains isolated from retail chickens, turkey (*), pork (†), beef (‡), and human clinical isolate* C. jejuni *81-176 for the ability to adhere to and invade T84 cells. Data were plotted as percentages of the starting viable inoculum. Means (±SD) of percentages of adherent and invaded bacteria are provided from three independent experiments. (B) The presence of putative virulence genes* flaA, cadF, ciaB, pldA, virB11, cdtA, cdtB, *and* cdtC *in the genomes and plasmids of* Campylobacter *isolates was evaluated by PCR using two different pairs of primers for each gene. Some genes (*) were detected by only one of the two pairs of PCR primers.*

exceptions. *Campylobacter* isolates C114, J385, and C49 had strong adherence but poor invasion abilities, whereas J237, J5, and J57 had relatively low adherence but moderate invasion abilities. These results suggest that the invasion abilities of *Campylobacter* retail meat isolates are associated with their adherent abilities, but the adherence of *Campylobacter* to the host epithelium does not necessarily lead to invasion.

Presence of the putative virulence genes. Even though the genes that determine the infectivity of *Campylobacter* have not been well studied, several genes are involved in *Campylobacter* adherence to and invasion of host cells. To test whether the relative adherence and invasion by the *Campylobacter* isolates are related to the presence of particular virulence genes, the presence of eight putative virulence genes in the genomic and plasmid DNA of the 43 retail meat isolates were determined by PCR using two different pairs of primers for each gene (Table 1). The majority of the *Campylobacter* isolates possessed seven of the eight virulence genes examined (Fig. 1B): *flaA* (100%), *cdtA* (100%), *cdtB* (100%), *cdtC* (100%), *ciaB* (91%), *cadF* (100%), and *pldA* (100%). However, the *virB11* gene of the pVir plasmid was not detected in most of the isolates except for chicken meat isolate C114 (Fig. 1B). All eight virulence genes were detected in the positive control strain *C. jejuni* 81-176. Genes *cdtA*, *cdtC*, *cadF*, and *pldA* in some of these isolates were detected by only one of the two pairs of primers used (Fig. 1B), possibly because of variability and random point mutations of the genes.

DISCUSSION

Campylobacter is one of the major foodborne pathogens and is widespread in retail meat, in particular poultry products. However, little is known about the pathogenicity of food-contaminating strains and the molecular basis of *Campylobacter* pathogenicity. In this study, we examined the ability of *Campylobacter* strains isolated from raw meat products to adhere to and invade cells of the T84 human intestinal epithelial line and determined the association between the presence of certain virulence genes and the adherence and invasion abilities of *Campylobacter* isolates from retail meats. Our results revealed that *Campylobacter*

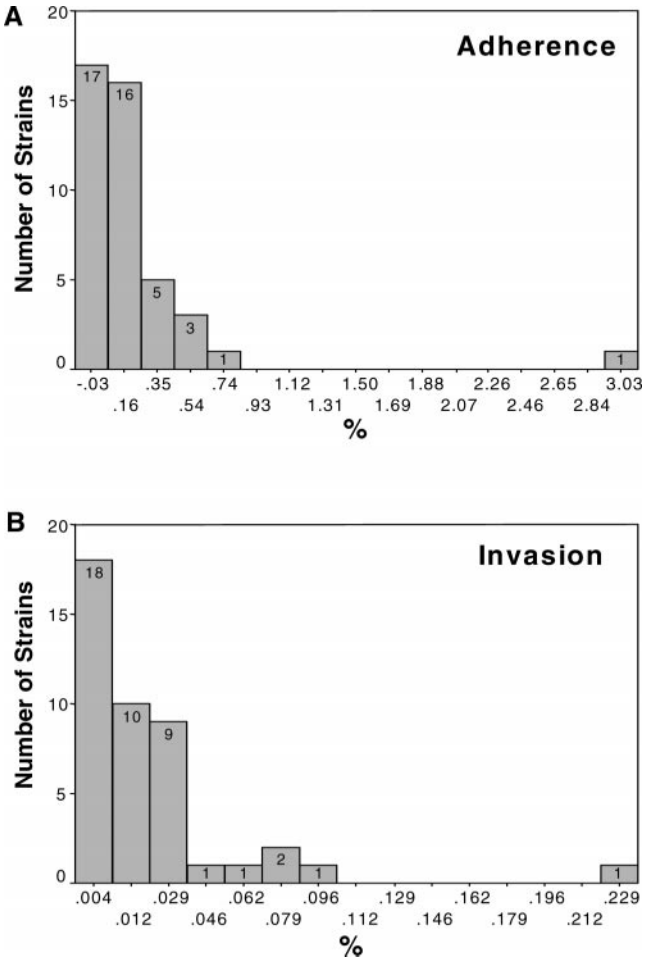


FIGURE 2. *Distribution of adherence and invasion abilities among 43* Campylobacter *strains isolated from retail meat. Experiments were performed as described for Figure 1A. The adherence (A) and invasion (B) efficiencies of* Campylobacter *retail meat isolates were expressed as percentages of the starting viable inoculum and plotted as a function of the number of strains.*

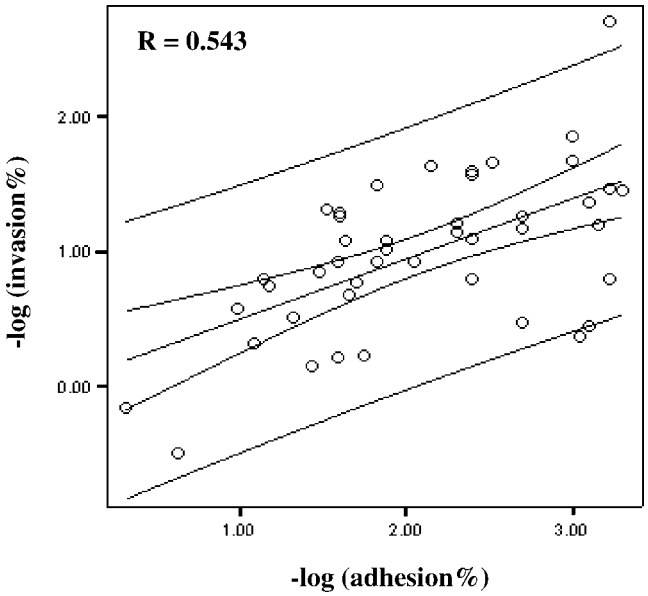


FIGURE 3. *Correlation between invasion and adherence abilities of 43* Campylobacter *strains isolated from retail meat was analyzed by linear regression using SPSS 10.0 software. The plot of −log(invasion %) versus −log(adherence %) of each strain is shown. The central line represents the regression line: [−log(adhesion %)] = 0.06 + 0.45[−log(invasion %)]; the inner lines near the central line represent the 95% mean prediction interval, and the outer lines represent 95% individual prediction intervals.*

strains with adherence and invasion abilities similar to those of the human clinical strain *C. jejuni* 81-176 were present in retail meat. Six of 43 tested isolates had invasion abilities close to those of *C. jejuni* 81-176, findings similar to those of a recent study in which 12 of 42 isolates from beef carcasses and from fecal samples collected from food and companion animals were capable of invading epithelial cells *(30).* The adherence and invasion abilities of the retail meat isolates varied considerably, from levels similar to that of *C. jejuni* 81-176 to 1,000-fold lower than that of *C. jejuni* 81-176. However, more than half of the retail meat isolates tested had an invasion efficiency 100-fold lower than that of *C. jejuni* 81-176, indicating that not all *Campylobacter* strains that contaminate meat products are able to effectively invade human intestinal epithelial cells.

Because animal models that completely mimic *Campylobacter* infections in humans are not available, the cell culture model using human colonic epithelial cells is a useful tool for evaluating the abilities of *Campylobacter* food isolates to adhere to and invade the human intestinal epithelium. The T84 cell line has been widely used for studies of pathogenicity of many human enteric pathogens, including *Salmonella* Typhimurium, *Helicobacter pylori,* and enteropathogenic *E. coli (7, 31, 37).* Even though the process of *Campylobacter* adhering to and invading T84 human intestinal epithelial cells in culture does not exactly mimic the process in vivo, the cell culture model allowed us to determine the relative adherence and invasion abilities of the retail meat isolates in comparison to the well-studied human clinical strain *C. jejuni* 81-176. To ensure that the cell culture model was functioning properly, we carried out inoculation dose and time course studies (data not shown), which indicated that the binding of *C. jejuni* to T84 cells was time dependent and saturable. Even though a multiplicity of infection of about 10 instead of 100 was used in this study, the invasion and adherence levels of the human clinical strain *C. jejuni* 81-176 reported here are similar to those of previously published studies *(23, 34, 45).*

Colonization or adherence of microbial pathogens to mucosal surfaces is the primary step of infection and appears to be a prerequisite for invasion in most cases *(1, 21, 22, 49).* In this study, we analyzed the relationship between adhesion efficiency and invasion efficiency in *Campylobacter* retail meat isolates using statistical tests. A positive correlation between adhesion and invasion was found, which suggests that host invasion efficiency of *Campylobacter* is at least partially dependent on its adherence ability. This correlation was not found in all of the tested strains. Some of the isolates that adhered to T84 cells did not efficiently invade these cells. Thus, adherence of *Campylobacter* may facilitate invasion into host cells but does not necessarily lead to invasion.

Bacterial virulence is multifactorial and is affected by the expression of virulence genes. To determine the relationship between the presence of virulence genes in *Campylobacter* retail meat isolates and the ability of these isolates to adhere to and invade human intestinal epithelial cells, the prevalence of the putative virulence genes *flaA*, *cadF*, *ciaB*, *pldA*, and *virB11* and the toxin genes *cdtA*, *cdtB*, and *cdtC* among the 43 *Campylobacter* retail meat isolates was determined by PCR. Even though cytolethal distending toxin has not been shown to be directly involved in host epithelial invasion, its role in inducing proinflammatory cytokine secretion by host epithelial cells *(18)* and inhibiting cell cycles *(48)* could influence the invasion and adherence ability of *Campylobacter*. Using two different pairs of PCR primers that target conserved sequences for each gene, we detected six of the eight putative virulence genes in all of the 43 *Campylobacter* isolates. The gene *ciaB* was undetectable in four isolates with very low invasion abilities. In some of the retail meat isolates, the putative virulence genes were detected with one of the two PCR primer sets used, suggesting the presence of variability and/or random mutations in these genes. The high prevalence of these genes in *Campylobacter* retail meat isolates with wide ranges of adherence and invasion efficiencies also suggests that additional genes may be involved in these processes. Our finding is consistent with previous studies that revealed the high prevalence of the same seven putative virulence genes in *Campylobacter* strains isolated from Danish turkeys, pigs, and cattle *(4, 5)*. In addition to *C. jejuni* 81-176, only one of the 43 retail meat isolates (which had an invasion ability 1,000 times lower than that of *C. jejuni* 81-176) was positive for the *virB11* gene. Bang et al. *(4, 5)* also reported a relatively low prevalence for the *virB11* gene in *Campylobacter* isolates compared with the prevalence of other putative virulence genes tested. The prevalence of *virB11* among food isolates has not been well studied. We did not find a general correlation between the invasion efficiency of *Campylobacter* retail meat isolates and the putative virulence genes present in genomic DNA. There are several possible explanations. First, although some of the poorly invasive strains contain most of virulence genes, they may fail to express these genes during interaction with host cells or they may express inactive variants of these genes. Second, virulence genes may have redundant and overlapping functions; thus, the absence of one or two of these genes may not significantly affect the invasion ability of *Campylobacter*. Third, in addition to these eight virulence genes, additional genes may be required for host invasion. Further studies using DNA microarrays and mutagenesis are required to identify additional virulence genes of *Campylobacter*, to determine which virulence genes are expressed upon host cell contact, to examine the variability of virulence genes, and to explore how different virulence genes work together to establish infection.

ACKNOWLEDGMENTS

We thank Dr. Beilei Ge for technical assistance. This study was supported in part by a grant to Dr. Wenxia Song from the Joint Institute for Food Safety and Applied Nutrition.

REFERENCES

1. Allos, B. M., and M. J. Blaser. 1995. *Campylobacter jejuni* and the expanding spectrum of related infections. *Clin. Infect. Dis.* 20:1092–1099; quiz, 1100–1101.
2. Altekruse, S. F., N. J. Stern, P. I. Fields, and D. L. Swerdlow. 1999. *Campylobacter jejuni*—an emerging foodborne pathogen. *Emerg. Infect. Dis.* 5:28–35.
3. Bacon, D. J., R. A. Alm, L. Hu, T. E. Hickey, C. P. Ewing, R. A. Batchelor, T. J. Trust, and P. Guerry. 2002. DNA sequence and mutational analyses of the pVir plasmid of *Campylobacter jejuni* 81-176. *Infect. Immun.* 70:6242–6250.
4. Bang, D. D., B. Borck, E. M. Nielsen, F. Scheutz, K. Pedersen, and M. Madsen. 2004. Detection of seven virulence and toxin genes of *Campylobacter jejuni* isolates from Danish turkeys by PCR and cytolethal distending toxin production of the isolates. *J. Food Prot.* 67: 2171–2177.
5. Bang, D. D., E. M. Nielsen, F. Scheutz, K. Pedersen, K. Handberg, and M. Madsen. 2003. PCR detection of seven virulence and toxin genes of *Campylobacter jejuni* and *Campylobacter coli* isolates from Danish pigs and cattle and cytolethal distending toxin production of the isolates. *J. Appl. Microbiol.* 94:1003–1014.
6. Black, R. E., M. M. Levine, M. L. Clements, T. P. Hughes, and M. J. Blaser. 1988. Experimental *Campylobacter jejuni* infection in humans. *J. Infect. Dis.* 157:472–479.
7. Corthesy-Theulaz, I., N. Porta, E. Pringault, L. Racine, A. Bogdanova, J. P. Kraehenbuhl, A. L. Blum, and P. Michetti. 1996. Adhesion of *Helicobacter pylori* to polarized T84 human intestinal cell monolayers is pH dependent. *Infect. Immun.* 64:3827–3832.
8. Cover, T. L., G. I. Perez-Perez, and M. J. Blaser. 1990. Evaluation of cytotoxic activity in fecal filtrates from patients with *Campylobacter jejuni* or *Campylobacter coli* enteritis. *FEMS Microbiol. Lett.* 58:301–304.
9. Datta, S., H. Niwa, and K. Itoh. 2003. Prevalence of 11 pathogenic genes of *Campylobacter jejuni* by PCR in strains isolated from humans, poultry meat and broiler and bovine faeces. *J. Med. Microbiol.* 52:345–348.
10. de Melo, M. A., G. Gabbiani, and J. C. Pechere. 1989. Cellular events and intracellular survival of *Campylobacter jejuni* during infection of HEp-2 cells. *Infect. Immun.* 57:2214–2222.
11. de Melo, M. A., and J. C. Pechere. 1988. Effect of mucin on *Campylobacter jejuni* association and invasion on HEp-2 cells. *Microb. Pathog.* 5:71–76.
12. Everest, P. H., H. Goossens, J. P. Butzler, D. Lloyd, S. Knutton, J. M. Ketley, and P. H. Williams. 1992. Differentiated Caco-2 cells as a model for enteric invasion by *Campylobacter jejuni* and *C. coli*. *J. Med. Microbiol.* 37:319–325.
13. Eyigor, A., K. A. Dawson, B. E. Langlois, and C. L. Pickett. 1999. Cytolethal distending toxin genes in *Campylobacter jejuni* and *Campylobacter coli* isolates: detection and analysis by PCR. *J. Clin. Microbiol.* 37:1646–1650.
14. Friedman, C. R., J. Neimann, H. C. Wegener, and R. V. Tauxe. 2000. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations, p. 121–138. *In* I. Nachamkin and M. J. Blaser (ed.), *Campylobacter*, 2nd ed. ASM Press, Washington, D.C.
15. Grant, C. C., M. E. Konkel, W. Cieplak, Jr., and L. S. Tompkins. 1993. Role of flagella in adherence, internalization, and translocation of *Campylobacter jejuni* in nonpolarized and polarized epithelial cell cultures. *Infect. Immun.* 61:1764–1771.
16. Griffiths, P. L., and R. W. Park. 1990. Campylobacters associated with human diarrhoeal disease. *J. Appl. Bacteriol.* 69:281–301.
17. Guerry, P., P. M. Pope, D. H. Burr, J. Leifer, S. W. Joseph, and A. L. Bourgeois. 1994. Development and characterization of *recA* mutants of *Campylobacter jejuni* for inclusion in attenuated vaccines. *Infect. Immun.* 62:426–432.
18. Hickey, T. E., A. L. McVeigh, D. A. Scott, R. E. Michielutti, A. Bixby, S. A. Carroll, A. L. Bourgeois, and P. Guerry. 2000. *Campylobacter jejuni* cytolethal distending toxin mediates release of interleukin-8 from intestinal epithelial cells. *Infect. Immun.* 68:6535–6541.

19. Hu, L., and D. J. Kopecko. 1999. *Campylobacter jejuni* 81-176 associates with microtubules and dynein during invasion of human intestinal cells. *Infect. Immun.* 67:4171–4182.
20. Johnson, W. M., and H. Lior. 1984. Toxins produced by *Campylobacter jejuni* and *Campylobacter coli*. *Lancet* 1:229–230.
21. Ketley, J. M. 1995. The 16th C. L. Oakley lecture. Virulence of *Campylobacter* species: a molecular genetic approach. *J. Med. Microbiol.* 42:312–327.
22. Ketley, J. M. 1997. Pathogenesis of enteric infection by *Campylobacter*. *Microbiology* 143:5–21.
23. Konkel, M. E., M. D. Corwin, L. A. Joens, and W. Cieplak. 1992. Factors that influence the interaction of *Campylobacter jejuni* with cultured mammalian cells. *J. Med. Microbiol.* 37:30–37.
24. Konkel, M. E., S. A. Gray, B. J. Kim, S. G. Garvis, and J. Yoon. 1999. Identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* based on the *cadF* virulence gene and its product. *J. Clin. Microbiol.* 37:510–517.
25. Konkel, M. E., and L. A. Joens. 1989. Adhesion to and invasion of HEp-2 cells by *Campylobacter* spp. *Infect. Immun.* 57:2984–2990.
26. Konkel, M. E., B. J. Kim, V. Rivera-Amill, and S. G. Garvis. 1999. Identification of proteins required for the internalization of *Campylobacter jejuni* into cultured mammalian cells. *Adv. Exp. Med. Biol.* 473:215–224.
27. Konkel, M. E., D. J. Mead, S. F. Hayes, and W. Cieplak, Jr. 1992. Translocation of *Campylobacter jejuni* across human polarized epithelial cell monolayer cultures. *J. Infect. Dis.* 166:308–315.
28. Korlath, J. A., M. T. Osterholm, L. A. Judy, J. C. Forfang, and R. A. Robinson. 1985. A point-source outbreak of campylobacteriosis associated with consumption of raw milk. *J. Infect. Dis.* 152:592–596.
29. Lara-Tejero, M., and J. E. Galan. 2001. CdtA, CdtB, and CdtC form a tripartite complex that is required for cytolethal distending toxin activity. *Infect. Immun.* 69:4358–4365.
30. Lee, M. K., S. J. Billington, and L. A. Joens. 2004. Potential virulence and antimicrobial susceptibility of *Campylobacter jejuni* isolates from food and companion animals. *Foodborne Pathog. Dis.* 1: 223–230.
31. McCormick, B. A., S. P. Colgan, C. Delp-Archer, S. I. Miller, and J. L. Madara. 1993. *Salmonella typhimurium* attachment to human intestinal epithelial monolayers: transcellular signalling to subepithelial neutrophils. *J. Cell Biol.* 123:895–907.
32. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5:607–625.
33. Millar, B. C., X. Jiru, J. E. Moore, and J. A. Earle. 2000. A simple and sensitive method to extract bacterial, yeast and fungal DNA from blood culture material. *J. Microbiol. Methods* 42:139–147.
34. Monteville, M. R., and M. E. Konkel. 2002. Fibronectin-facilitated invasion of T84 eukaryotic cells by *Campylobacter jejuni* occurs preferentially at the basolateral cell surface. *Infect. Immun.* 70:6665–6671.
35. Monteville, M. R., J. E. Yoon, and M. E. Konkel. 2003. Maximal adherence and invasion of INT 407 cells by *Campylobacter jejuni* requires the CadF outer-membrane protein and microfilament reorganization. *Microbiology* 149:153–165.
36. Morris, J. G., Jr. 1996. Current trends in human diseases associated with foods of animal origin. *J. Am. Vet. Med. Assoc.* 209:2045–2047.
37. Nataro, J. P., S. Hicks, A. D. Phillips, P. A. Vial, and C. L. Sears. 1996. T84 cells in culture as a model for enteroaggregative *Escherichia coli* pathogenesis. *Infect. Immun.* 64:4761–4768.
38. Newell, D. G., H. McBride, and J. M. Dolby. 1985. Investigations on the role of flagella in the colonization of infant mice with *Campylobacter jejuni* and attachment of *Campylobacter jejuni* to human epithelial cell lines. *J. Hyg. (Lond.)* 95:217–227.
39. Oelschlaeger, T. A., P. Guerry, and D. J. Kopecko. 1993. Unusual microtubule-dependent endocytosis mechanisms triggered by *Campylobacter jejuni* and *Citrobacter freundii*. *Proc. Natl. Acad. Sci. USA* 90:6884–6888.
40. Pei, Z., C. Burucoa, B. Grignon, S. Baqar, X. Z. Huang, D. J. Kopecko, A. L. Bourgeois, J. L. Fauchere, and M. J. Blaser. 1998. Mutation in the *peb1A* locus of *Campylobacter jejuni* reduces interactions with epithelial cells and intestinal colonization of mice. *Infect. Immun.* 66:938–943.
41. Purdy, D., C. M. Buswell, A. E. Hodgson, K. McAlpine, I. Henderson, and S. A. Leach. 2000. Characterisation of cytolethal distending toxin (CDT) mutants of *Campylobacter jejuni*. *J. Med. Microbiol.* 49:473–479.
42. Rivera-Amill, V., B. J. Kim, J. Seshu, and M. E. Konkel. 2001. Secretion of the virulence-associated *Campylobacter* invasion antigens from *Campylobacter jejuni* requires a stimulatory signal. *J. Infect. Dis.* 183:1607–1616.
43. Rousset, M. 1986. The human colon carcinoma cell lines HT-29 and Caco-2: two in vitro models for the study of intestinal differentiation. *Biochimie* 68:1035–1040.
44. Ruiz-Palacios, G. M., E. Escamilla, and N. Torres. 1981. Experimental *Campylobacter* diarrhea in chickens. *Infect. Immun.* 34:250–255.
45. Russell, R. G., and D. C. Blake, Jr. 1994. Cell association and invasion of Caco-2 cells by *Campylobacter jejuni*. *Infect. Immun.* 62: 3773–3779.
46. Russell, R. G., M. O'Donnoghue, D. C. Blake, Jr., J. Zulty, and L. J. DeTolla. 1993. Early colonic damage and invasion of *Campylobacter jejuni* in experimentally challenged infant *Macaca mulatta*. *J. Infect. Dis.* 168:210–215.
47. Wassenaar, T. M., N. M. Bleumink-Pluym, and B. A. van der Zeijst. 1991. Inactivation of *Campylobacter jejuni* flagellin genes by homologous recombination demonstrates that *flaA* but not *flaB* is required for invasion. *EMBO J.* 10:2055–2061.
48. Whitehouse, C. A., P. B. Balbo, E. C. Pesci, D. L. Cottle, P. M. Mirabito, and C. L. Pickett. 1998. *Campylobacter jejuni* cytolethal distending toxin causes a G2-phase cell cycle block. *Infect. Immun.* 66:1934–1940.
49. Wooldridge, K. G., and J. M. Ketley. 1997. *Campylobacter*–host cell interactions. *Trends Microbiol.* 5:96–102.
50. Yanagawa, Y., M. Takahashi, and T. Itoh. 1994. The role of flagella of *Campylobacter jejuni* in colonization in the intestinal tract in mice and the cultured-cell infectivity. *Nippon Saikingaku Zasshi* 49:395–403. (In Japanese.)
51. Zhao, C., B. Ge, J. De Villena, R. Sudler, E. Yeh, S. Zhao, D. G. White, D. Wagner, and J. Meng. 2001. Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the greater Washington, D.C., area. *Appl. Environ. Microbiol.* 67:5431–5436.
52. Ziprin, R. L., C. R. Young, L. H. Stanker, M. E. Hume, and M. E. Konkel. 1999. The absence of cecal colonization of chicks by a mutant of *Campylobacter jejuni* not expressing bacterial fibronectin-binding protein. *Avian Dis.* 43:586–589.