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Genotypic Analysis of the Virulence and Antibiotic Resistance Genes in *Campylobacter* species *in silico*

Nusrat Nahar* and Ridwan Bin Rashid

Computational Chemistry and Bioinformatics Laboratory, Department of Pharmacy, State University of Bangladesh, Dhaka, Bangladesh

Abstract

Campylobacter species is responsible for 400-500 million diarrhea cases worldwide every year. Emergence of antibiotic resistance has further complicated the scenario. A wide range of virulence factors and resistance genes are present in *Campylobacter* species and it is hypothesized there are genotypic variations in the prevalence of these genes. The study was conducted to investigate the presence of virulence and antibiotic resistance genes as well as to investigate difference in prevalence rate based on genotype through *in silico* tools. Among 26 species studied, sixteen isolates (61.54%) had the *cdtB* gene that breaks the double helix bonds. The *cdtA* genes were detected in ten (38.46%) *C. jejuni* strains while fifty percent (n=13) isolates harbored the *cdtC* genes. Ten isolates that harboured all three adjacent *cdt* genes were most toxigenic. The lipo-oligosaccharides associated genes, *cgtB* and *wlaN*, responsible for β-1,3 galactosyltransferase production, were found in 7.69% and 30.77% of the isolates, respectively. About 57.69% isolates expressed *waaC* genes. Invasion protein *ciaB*, outer membrane phospholipase A *pldA* and IV secretory protein *virB11* were found in 53.85%, 34.62% and 7.69% of the isolates, respectively. Six isolates (23.08%) expressed both *tetO* and *tetA* genes while one isolate expressed only *tetA* resistance gene. Seven isolates (26.92%) had changes in *gyrB* genes that conferred the fluoroquinolone resistance. *In silico* PFGE typing found that genotype 3 contained all the virulence genes except *cgtB* gene while genotype 3 and 4 contained mutated *gyrB* gene. Genotype 1 and 5 contained no virulence and antibiotic resistance genes. Our data helps to predict the possibility of the presence of virulence and antibiotic resistance genes and helps to select appropriate antibiotic that are more efficacious.

Keywords: Antibiotic resistance genes; *Campylobacter;* Genotype; PCR; PFGE; Virulence genes

Introduction

Gram-negative *Campylobacter* species is responsible for traveler's diarrhea and gastroenteritis in humans [1,2]. *Campylobacter* is responsible for 400-500 million diarrhea cases worldwide every year [3]. Guillain–Barré syndrome is caused by *C jejuni* [4] but virulence mechanisms are not well understood. Several studies found that contaminated food, raw milk, water, vegetables, seafood, atmospheric modified packed meat transmitted the zoonotic pathogen *Campylobacter* to humans during consumption or handling process [1,2,5]. Several investigations found that main source of food-borne human campylobacteriosis is poultry and poultry products [6,7]. During slaughtering, the intestinal tract of healthy birds and raw meat are contaminated with thermophilic *Campylobacter* species. In slaughter house, faeces and processing facilities during the evisceration process contaminated swine carcasses that ultimately leads to contaminated food products [8-10].

The mechanism of *Campylobacter* gastroenteritis in humans is not well known that limits the prevention of campylobacteriosis. Virulence factors such as motility and adherence of bacteria to the intestinal mucosa, invasion of enterocyte and toxin production may contribute to the pathogenicity of campylobacteriosis infections [11,12]. The most common virulence factor in *Campylobacter* species, the Cytolethal Distending Toxin (CDT) causes cellular distension which eventually leads to cell death [13]. The CDT has 3 subunits namely CdtA, CdtB, CdtC. The CdtB is the active subunit whereas CdtA and CdtC makes up the B subunit responsible for binding to susceptible cells [14]. Active toxin component of *cdtB* gene disrupted the double helix bonds in the nucleus and blocked the cell cycle [15,16]. The *wlaN*, *cgtB* and *waaC* are LOS (lipo-oligosaccharides) associated genes while *wlaN* and *cgtB* are involved in β-1,3 galactosyltransferase production [17]. These two genes are associated with *waaC* gene (which encodes heptosyltransferase I) and connected with the Guillain-Barre' and Miller-Fischer syndromes [18,19]. GBS and other neuropathic conditions are caused because of molecular mimicry of *Campylobacter* lipooligosaccharide (LOS) with the carbohydrate moiety of gangliosides. The *waaC* gene, which encodes heptosyltransferase I, is responsible for transferring the first l-*glycero*-d-*manno*-heptose residue to the inner core of LOS [20]. The *wlaN* gene, which encodes a beta-1,3 galactosyltransferase, is responsible for biosynthesis GM1-like structure [18] whereas *cgtB* (which encodes another beta-1,3 galactosyltransferase) catalyzes the biosynthesis of the carbohydrate moieties analogous to GM2 [18,21].

Invasion protein (*ciaB*), outer membrane phospholipase A (*pldA*), and type IV secretory protein (*virB11*) genes associated with bacterial invasion on epithelial cells, were found by previous study [22] but their functions are not well known. Phospholipase A, *pldA* was found to be expressed in invasive strains [23].

Several studies found that multidrug-resistant *Campylobacter* has increased worldwide due to misuse of antibiotics [24,25]. For the treatment of systemic campylobacteriosis infections, other antibiotics such as gentamicin, tetracycline and ampicillin have been used [26,27].

***Corresponding author:** Nusrat Nahar, Computational Chemistry and Bioinformatics Laboratory, Department of Pharmacy, State University of Bangladesh, Dhaka, Bangladesh, Tel: 09613782338; E-mail: nusratnahar17@gmail.com

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Fluoroquinolones, followed by tetracyclines, are the most commonly used antibiotics in the poultry industry [28] where *Campylobacter* is ubiquitous. It can be inferred that over use of tetracyclines and fluoroquinolones have contributed to their resistance. Mutations in *gyrA* and *gyrB* regions of DNA gyrase and been held accountable for resistance against fluoroquinolones such as ciprofloxacin [29]. Majority of fluoroquinolone resistance of *Campylobacter* is developed by amino acid substitution at Thr-86 to Ilu though alternative mutation at Asp-90 and double mutation at Thr-86 and Pro-104 were also reported [30].

Resistance to tetracycline might be due to efflux, enzymatic alternation of antibiotic or by ribosomal protection. The *tetA* gene encodes efflux protein associated with pumping out tetracyclines out of the cell [31]. The tetO causes resistance by ribosomal protection whereas tetX gene encodes enzymes responsible for target modification [31]. Scientists identified that tetO protein recognized and bound with an open A site of the bacterial ribosome and conformational changes occurred that released bound tetracycline molecules [32]. Previous findings found that tetO also inhibited tRNA accommodation into the ribosomal A site and inhibited protein elongation phase [33].

Pulsed Field Gel Electrophoresis (PFGE) is considered as the gold standard for genotyping [34] and *in silico* data matched to that obtained in conventional method [35]. Our data helps to predict virulence and antimicrobial resistance profile of twenty-six *Campylobacter* species through *in silico* tools and also analyzes how pulsed-field gel electrophoresis (PFGE) typing distributed the virulence and resistance genes within the genotypes. The virulence and resistance profile of future isolates with known genotype can be predicted according to our data.

Materials and Methods

Strains used in the study

Strains used in the study are summarized in Table 1. All strains were of human/ animal origin.

Primers used in the study

Primers used for detection of virulence and antibiotic resistance genes are summarized in Tables 2 and 3 [36-43].

PCR amplification

In silico PCR amplification was done in the website http://insilico.ehu.eus/PCR/ [35,44].

PFGE digestion

An online software http://insilico.ehu.es/digest/wasdesigned for *insilico* pulsed-field gel electrophoresis (PFGE) digestion [35,44]. Restriction enzyme *Kpn*I recognized the restriction sequenceG_GTAC'C of *Campylobacter* species. Dendrogram construction was done in the website.

Results and Discussion

Genetic diversity of isolates

A total of 26 isolates were subjected to *in silico* pulsed-field gel electrophoresis (PFGE) analysis with *Kpn*I restriction digestion that recognized the sequence G_GTAC'C of *Campylobacter* species. Band fragments were separated in 1.2% agarose gel and lambda ladder compared the band size. *In silico* PFGE typing grouped 26 isolates into five genotypes using 80% as a cutoff value (Figure 1). Genotype 3 was most prevalent followed by genotype 4 and 2. Fifty percent (n=13) isolates were present in genotype 3 whereas genotype 4 contained about 26.92% (n=7) of the isolates. Genotype 1 and 3 and harboured about 3.85% of the isolates (Figure 2).

Serial number	Isolate
1	NC_022660 *Campylobacter coli* 15-537360
2	NC_022132 *Campylobacter coli* 76339
3	NC_022347 *Campylobacter coli* CVM N29710
4	NC_009802 *Campylobacter concisus* 13826
5	NC_009715 *Campylobacter curvus* 525.92
6	NC_008599 *Campylobacter fetus* subsp. *fetus* 82-40
7	NC_009714 *Campylobacter hominis* ATCC BAA-381
8	NC_021834 *Campylobacter jejuni* 32488
9	NC_022529 *Campylobacter jejuni* 4031
10	NC_003912 *Campylobacter jejuni* RM1221
11	NC_009707 *Campylobacter jejuni* subsp. *doylei* 269.97
12	NC_022362 *Campylobacter jejuni* subsp. *jejuni* 00-2425
13	NC_022352 *Campylobacter jejuni* subsp. *jejuni* 00-2426
14	NC_022351 *Campylobacter jejuni* subsp. *jejuni* 00-2538
15	NC_022353 *Campylobacter jejuni* subsp. *jejuni* 00-2544
16	NC_008787 *Campylobacter jejuni* subsp. *jejuni* 81-176
17	NC_009839 *Campylobacter jejuni* subsp. *jejuni* 81116
18	NC_017279 *Campylobacter jejuni* subsp. *jejuni* IA3902
19	NC_014802 *Campylobacter jejuni* subsp. *jejuni* ICDCCJ07001
20	NC_017280 *Campylobacter jejuni* subsp. *jejuni* M1
21	NC_002163 *Campylobacter jejuni* subsp. *jejuni* NCTC 11168
22	NC_018521 *Campylobacter jejuni* subsp. *jejuni* NCTC 11168-BN148
23	NC_018709 *Campylobacter jejuni* subsp. *jejuni* PT14
24	NC_017281 *Campylobacter jejuni* subsp. *jejuni* S3
25	NC_012039 *Campylobacter lari* RM2100
26	NC_022759 *Campylobacter* sp. 03-427

Table 1: Name of the isolate.

	Primer sequence (5'-3')	Amplicon size (bp)	References
cdtA	CCT TGT GAT GCA AGC AAT C ACA CTC CAT TTG CTT TCT G	370	[36]
cdtB	CAGAAAGCA AAT GGA GTG TT AGC TAA AAG CGG TGG AGT AT	620	[12]
cdtC	TTGGCATTATAGAAAATA CAG TT CGATGAGTTAAAACAAAAAGATA	182	[12]
cgtB	TTAAGAGCAAGATATGAAGGTG GCACATAGAGAACGCTACAA	562	[18]
wlaN	TGCTGGGTATACAAAGGTTGTG AATTTTGGATATGGGTGGGG	330	[37]
waaC	TAATGAAAATAGCAATTGTTCGT GATACAAAAATCACTTTTATCGA	971	[38]
virB11	GAACAGGAAGTGGAAAAACTAGC TTCCGCATTGGGCTATATG	708	[39]
pldA	AAG AGT GAG GCG AAA TTC CA GCA AGA TGG CAG GAT TAT CA	385	[40]
ciaB	TGC GAG ATT TTT CGA GAA TG TGC CCG CCT TAG AAC TTA CA	527	[40]
iam	GCGCAAATATTATCACCC TTCACGACTACTACTATGCGG	518	[41]

Table 2: Primers for the detection of virulence genes.

Genotypic distribution of Cytolethal Distending Toxin associated *cdt* genes

Toxigenic activity of *Campylobacter* species is determined by cytolethal distending toxin which is composed of the *cdt* gene cluster containing three adjacent genes (*cdtA*, *cdtB* and *cdtC*). It was found that

three *cdt* genes determined the functional activity of CDT toxin [15]. Previously researchers identified that *cdtA* and *cdtC* are responsible for binding to target whereas the *cdtB* encoded active subunit of the toxin [15,45]. Sixteen isolates (61.54%) had the *cdtB* gene with 620 bp PCR product (Figure 3). Only *Campylobacter jejuni* subsp. *doylei* 269.97 didn't harbour *cdtB* gene out of the isolates studied. The *cdtA* gene was detected in ten (38.46%) *C. jejuni* strains with 370 bp gene product (Figure 4) while fifty percent (n=13) isolates harboured the *cdtC* genes. These isolates gave a 182 bp amplicon (Figure 5). The present study found ten isolates that harboured all three adjacent *cdt* genes and these were considered as most toxigenic strains. Genotype 1 and 5 contained no *cdt* genes (Figure 6) and hence such isolates are unlikely to cause gastroenteritis. On the other hand, genotype 2, 3 and 4 contained all three *cdt* genes. All the isolates present in genotype 3 carried *cdtB* genes while about 61.54% and 76.92% isolates present in genotype 3 expressed *cdtA* and *cdtC* genes, respectively. About 28.58% in genotype 4 expressed *cdtB* and *cdtC* genes while 14.29% isolates harboured *cdtA* gene in genotype 4. Twenty-five percent isolates in genotype 2 carried all three *cdt* genes. It must be noted that cdtB alone can cause cytotoxicity [46]. Hence isolates with only *cdtB* gene can cause gastroenteritis irrespective of the *cdtA* and *cdtC* although potency of toxin might be compromised. Thus all isolates from genotype 3 will cause diarrhea.

Genotypic distribution of genes associated with Guillain–Barré syndrome

The genes *cgtB, wlaN* and *waaC* are associated with GBS and other neuropathic conditions through molecular mimicry of LOS with gangliosides. The *cgtB* gene was detected in only two isolates and

Gene	Primer sequence (5'-3')	Amplicon size (bp)	References
tetO	GGCGTTTTGTTTATGTGCG ATGGACAACCCGACAGAAGC	559	[42]
tetA	GCTCACGTTGACGCAGGAAAG ATC GTC ATT GTC CGT TAC	486	[43]
gyrA	ACGCAAGAGATGGTT GCTGCGATGCGTTATACTGA	270	[30]
gyrB	ATGGCAGCTAGAGGAAGAGA GTGATCCATCAACATCCGCA	382	[30]

Table 3: Primers for detection of antibiotic resistance genes.

Geno type	Gene prevalence (%)	Gene functions
1	None of the studied genes were present	Diarrhea associated genes *cdtA*=cytolethal distending toxin subunit A, *cdtB*=cytolethal distending toxin subunit B, *cdtC*=cytolethal distending toxin subunit C Genes associated with Guillain–Barré syndrome *cgtB=Campylobacter* beta1,3-galactosyltransferase B responsible for GM2 mimicry, *wlaN*=β-1,3 galactosyltransferase responsible GM1 mimicry, *waaC*=heptosyltransferase I. Adherence and invasion genes: *pldA*=phospholipase A *virB11*=plasmid pVir invasion-associated marker *ciaB=Campylobacter* invasion antigen B Genes associated with tetracycline resistance *tetA*=Tetracycline resistance protein A *tetO*=Tetracycline resistance protein O Genes associated with fluoroquinolone resistance *gyrA*=gyrA subunit of DNA gyrase *gyrB*=gyrB subunit of DNA gyrase
2	*cdtA=25* *cdtB=25* *cdtC=25* *ctgB=25* *wlaN=0* *waaC=25* *pldA=0* *virB11=25* *ciaB=25* *tetA=25* *tetO=25* *gyrA=0* *gyrB=25*	
3	*cdtA=61.54* *cdtB=100* *cdtC=76.92* *ctgB=0* *wlaN=61.54* *waaC=100* *pldA=61.54* *virB11=7.69* *ciaB=84.62* *tetA=30.77* *tetO=23.08* *gyrA=0* *gyrB=23.08*	
4	*cdtA=14.29* *cdtB=28.58* *cdtC=28.58* *ctgB=14.29* *wlaN=0* *waaC=14.29* *pldA=14.27* *virB11=0* *ciaB=28.58* *tetA=28.57* *tetO=28.57* *gyrA=0* *gyrB=42.86*	
5	None of the studied genes were present	

Table 4: Summary of the results.

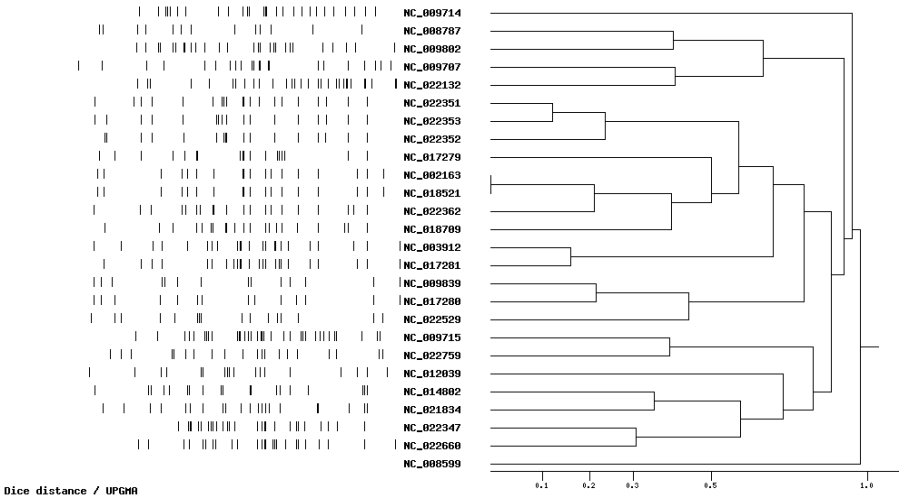

Figure 1: Phylogenetic diversity of *Campylobacter* species identified by PFGE analysis.

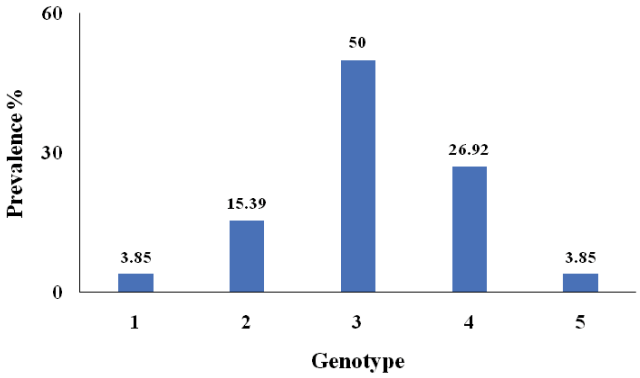

Figure 2: Prevalence of genotypes.

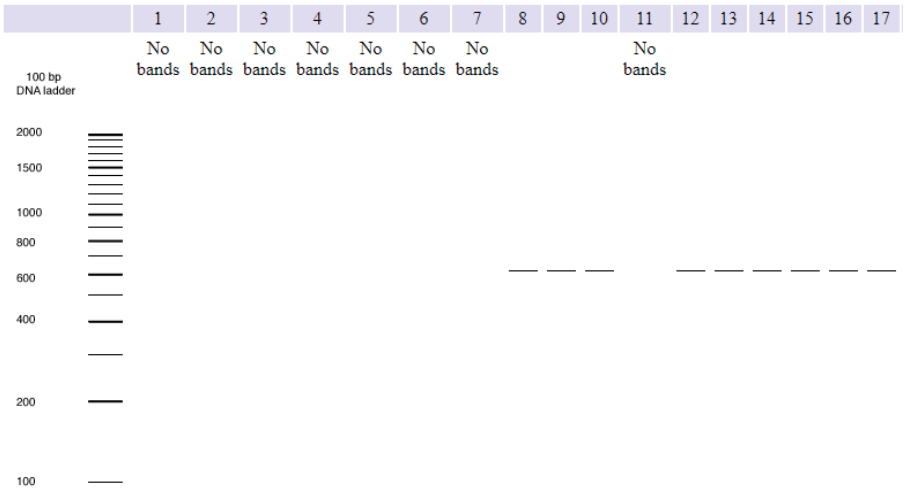

Figure 3: Detection of *cdtB* gene. Isolates harbouring the gene gives a 620 bp amplicon.

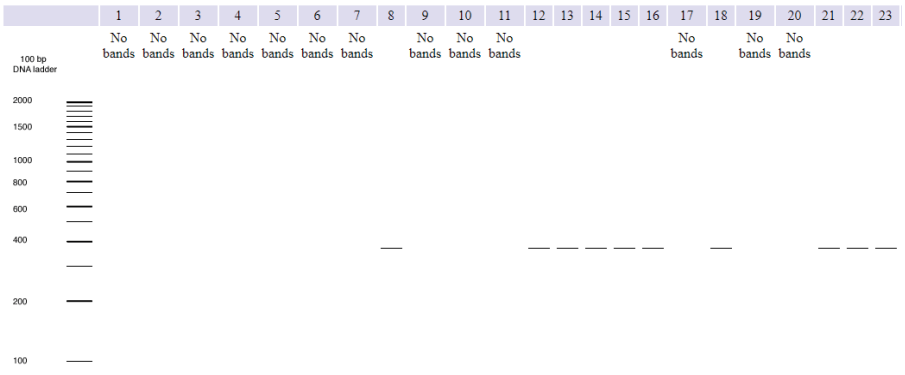

Figure 4: Detection of *cdtA* gene. Isolates harbouring the gene gives a 370 bp amplicon.

produced 562 bp gene product (Figure 7) while 30.77% isolates had the *wlaN* genes with 330 bp gene product (Figure 8). About 57.69% isolates expressed *waaC* genes and gave 971 bp gene product (Figure 9). These three LOS associated genes were found in only *C. jejuni* strains. The *wlaN* and *cgtB* genes were not found in the same isolates. Similar results were also found in previous study [17]. Not all LOS associated genes were present in same genotype (Figure 10). Twenty-five percent isolates in genotype 2 expressed *cgtB* and *waaC* genes. All the isolates in genotype 3 carried the *waaC* gene while about 61.54% isolates in genotype 3 expressed *wlaN* genes. About 14.29% isolates in genotype 4 carried both *cgtB* and *waaC* genes. Hence isolates from genotype 3 are more likely to exert their pathology through the mimicry of GM1 rather than GM2 whereas the reverse is true for isolates from genotype 2 and 4. All isolates from genotype 3 would exhibit heptosyltransferase

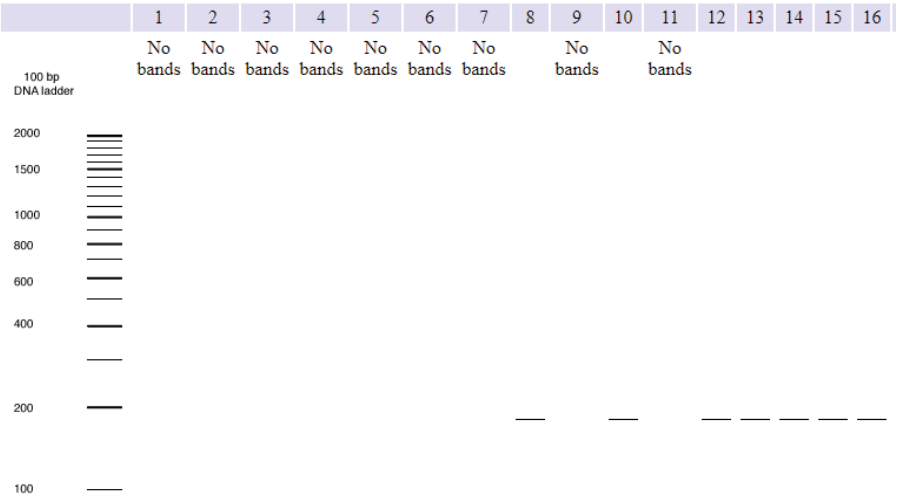

Figure 5: Detection of *cdtC* gene. Isolates harbouring the gene gives a 182 bp amplicon.

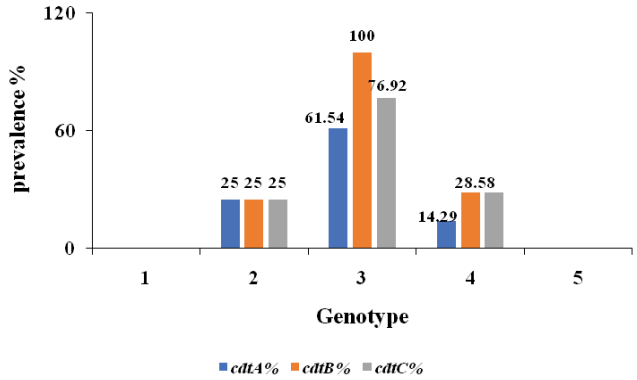

Figure 6: Genotypic distribution of *cdt* genes. Genes encoding virulence genes are as follows: *cdtA*: Cytolethal Distending Toxin A; *cdtB*: Cytolethal Distending Toxin B; *cdtC*: Cytolethal Distending Toxin C.

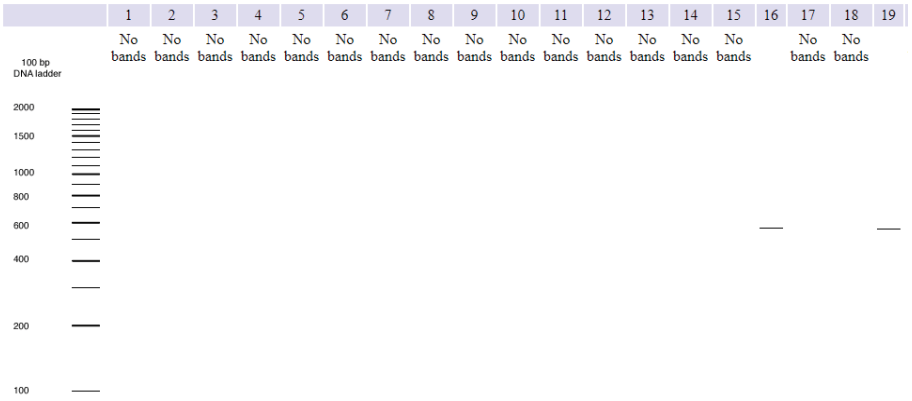

Figure 7: Detection of *cgtB* gene. Isolates harbouring the gene gives a 562 bp amplicon.

I activity and would transfer first l-*glycero*-d-*manno*-heptose residue to the inner core of LOS.

Genotypic distribution of adherence and invasion genes

Invasion-associated marker (*iam*), genetic marker of *Campylobacter* species, identified in diarrhea and symptom free patients, was reported by previous study [47]. Previous study found that clinical samples in children didn't harbor *iam* gene but isolates from older patients had the *iam* genes [48]. The present study found no invasion associated marker, *iam* gene. In Brazil, diarrhoeagenic *C. coli* isolates were found in children that had the *iam* gene while low prevalence of *iam* genes were found in *C. jejuni* strains [49]. Invasion protein (*ciaB*),

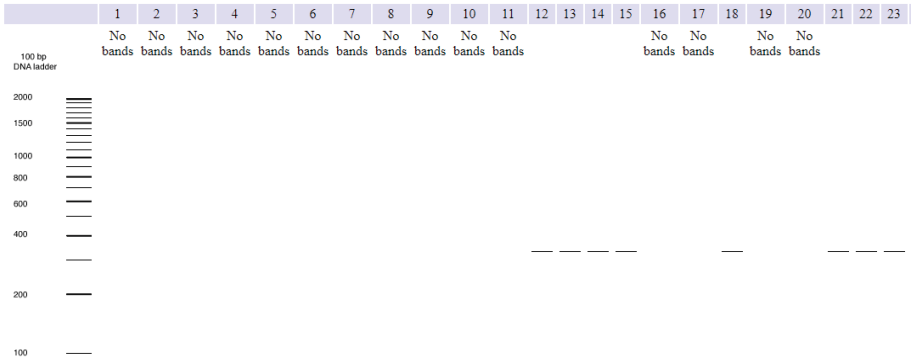

Figure 8: Detection of *wlaN* gene. Isolates harbouring the gene gives a 330 bp amplicon.

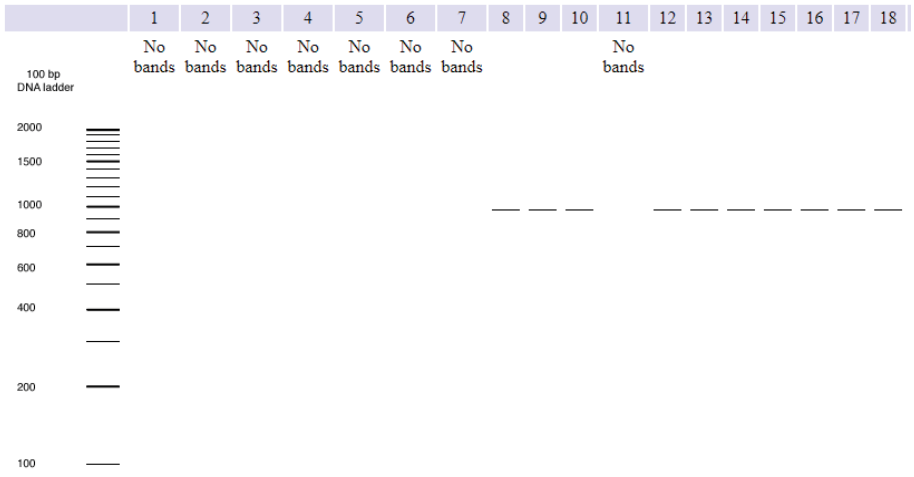

Figure 9: Detection of *waaC* gene. Isolates harbouring the gene gives a 971 bp amplicon.

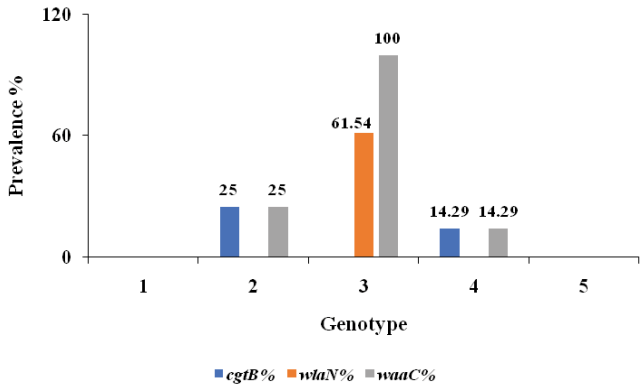

Figure 10: Genotypic distribution of *cgtB*, *wlaN* and *waaC* genes. Genes encoding virulence genes are as follows: *cgtB*: *Campylobacter* beta1; 3: Galactosyltransferase B Responsible for GM2 Mimicry; *wlaN*: β: 1, 3 Galactosyltransferase Responsible GM1 Mimicry; *waaC*: Heptosyltransferase I.

outer membrane phospholipase A (*pldA*), and IV secretory protein (*virB11*) genes associated with bacterial invasion on epithelial cells, were found by previous study [22] but their functions are not well known. Phospholipase A, *pldA* was found to be involved in expression of invasion [23]. A study found that cattle isolates didn't harbour phospholipase A, *pldA* gene while sheep samples were potentially more virulent since carrying more *iam* genes than that of cattle isolates [50]. About 34.62% isolates (n=9) had the *pldA* genes and produced 385 bp gene product (Figure 11) in *C. jejuni* strains. Previous study found that *pldA* gene was encountered in high number (88-100%) in broiler samples [51]. High percentage (91.7%) isolates from poultry feces contained *pldA* genes [52]. Several studies described that *ciaB* gene is associated with invasiveness and a play role in progression of the disease [22,53]. About 53.85% isolates (n=14) had the *ciaB* genes with 527 bp gene product (Figure 12). Previously 98.80% and 61% isolates were found to harbor *ciaB* and *pldA* genes, respectively

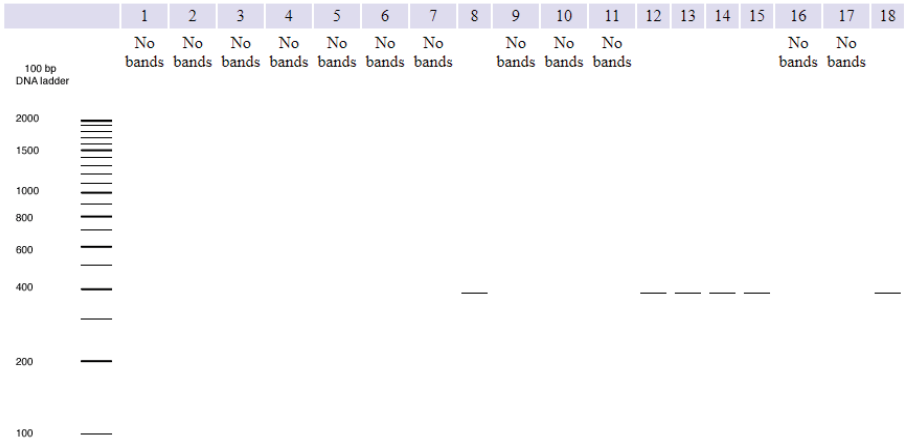

Figure 11: Detection of *pldA* gene. Isolates harbouring the gene gives a 385 bp amplicon.

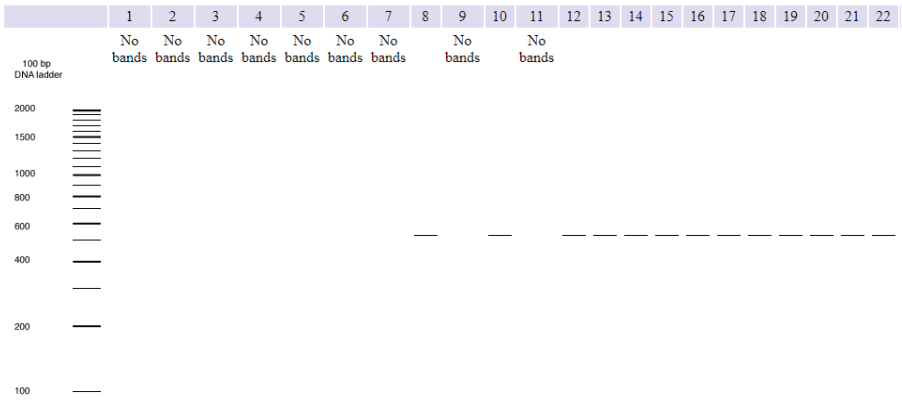

Figure 12: Detection of *ciaB* gene. Isolates harbouring the gene gives a 527 bp amplicon.

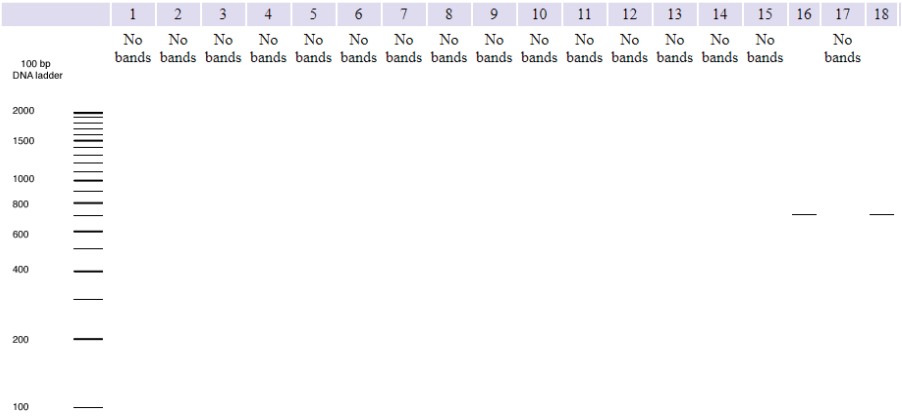

Figure 13: Detection of *virB11* gene. Isolates harbouring the gene gives a 708 bp amplicon.

[54]. Broiler meat samples and human strains equally harboured the plasmid associated virulence marker *virB11* gene that could invade the human intestine [55]. Researchers found that very small subset of *C. jejuni* strains carried *virB11* genes [39]. The *virB11* gene was detected in two isolates (7.69%) with 708 bp gene product (Figure 13). Nature of the plasmid and geographical differences are responsible for low level of *virB11* gene [52]. Several studies also found very low number of *virB11* genes in the tested isolates [51,56]. Genotype 3 contained all three genes described above (Figure 14). About 84.62% isolates in genotype 3 expressed *ciaB* genes while about 61.54% and 7.69% isolates in genotype 3 harboured *pldA* and *virB11* genes, respectively. About 14.29% isolates in genotype 4 carried *pldA* while about 28.58% isolates in genotype 4 carried *ciaB* genes. Twenty-five percent isolates in genotype 2 harboured *virB11* and *ciaB* genes. Hence it can be inferred

that invasion and adherence by isolates from genotype 2 are unlikely to be mediated by phospholipase A. The same can be stated invasion via virB11 invasion-associated marker for genotype 4.

Genotypic distribution of tetracycline resistance genes

Tetracycline resistance gene *tetO* was detected by using two primers named DMT1 and DMT2 as described previously [57]. Six isolates were found to harbor *tetO* resistance gene and gave 559 bp gene product (Figure 15). Hence the prevalence was 23.08%. Forty-six kilodalton membrane-bound efflux protein *tetA* exported tetracycline antibiotic from the cell [58]. Seven isolates (26.92%) expressed *tetA* gene with an approximate amplicon length of 486 bp (Figure 16). Six isolates expressed both *tetO* and *tetA* genes while *Campylobacter jejuni* subsp. *jejuni* M1 expressed only *tetA* resistance genes. Our data suggests that tetracycline resistance is more likely to be mediated due to *tetA* rather than *tetO*. Our data agrees with a study also found that more Kenyan isolates harboured *tetA* resistance genes rather than *tetO* [43]. Twenty-five percent isolates in genotype 2 carried *tetO* and *tetA*

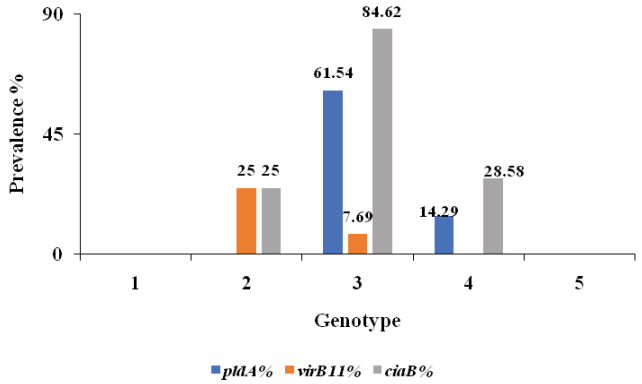

Figure 14: Genotypic distribution of *pldA*, *virB11* and *ciaB* genes. Genes encoding virulence genes are as follows: *pldA*: Phospholipase A; *virB11*: Plasmid; pVir: Invasion Associated Marker; *ciaB*: *Campylobacter* Invasion Antigen B.

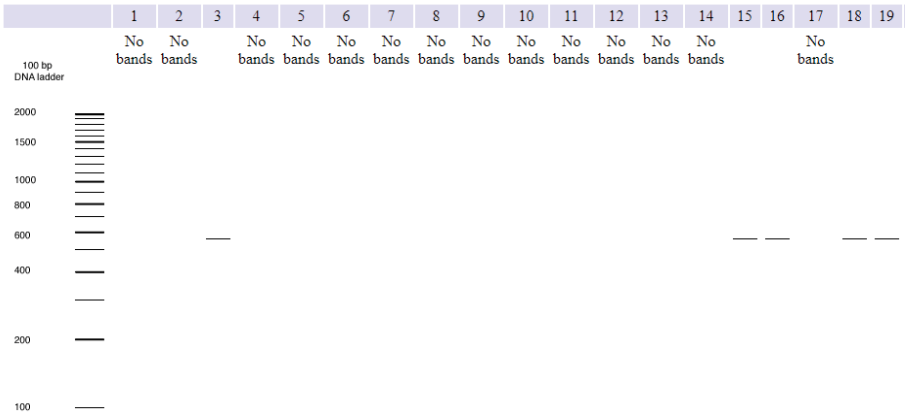

Figure 15: Detection of *tetO* gene. Isolates harbouring the gene gives a 559 bp amplicon.

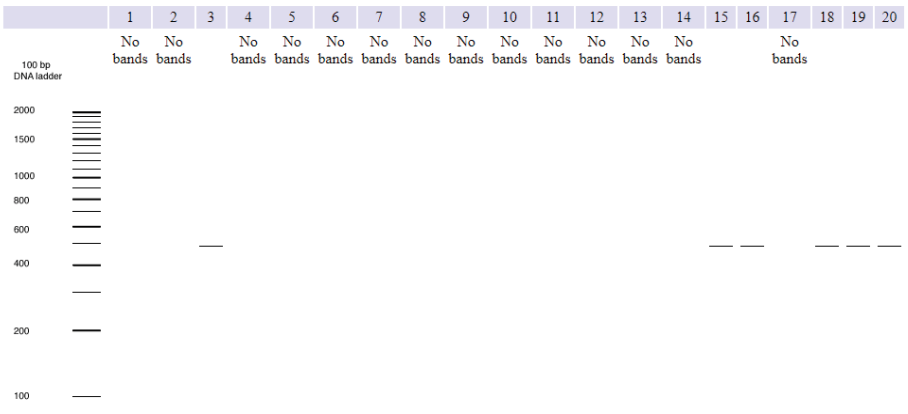

Figure 16: Detection of *tetA* gene. Isolates harbouring the gene gives a 486 bp amplicon.

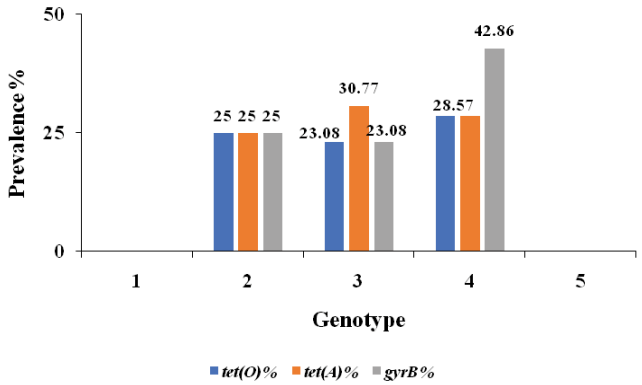

Figure 17: Genotypic distribution of tetracycline and fluoroquinolone resistance genes. Genes encoding resistance genes are as follows: *tetA*: Tetracycline Resistance Protein A; *tetO*: Tetracycline Resistance Protein O; *gyrB*: gyrB Subunit of DNA Gyrase.

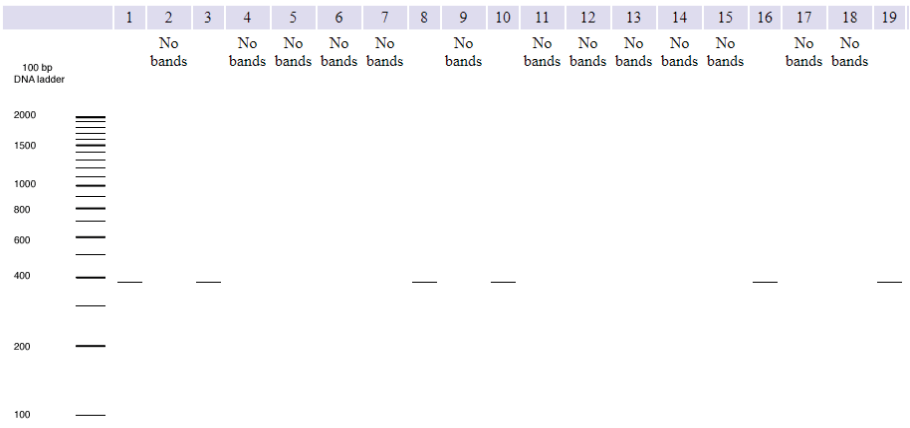

Figure 18: Detection of *gyrB* gene. Isolates harbouring the gene gives a 382 bp amplicon.

genes (Figure 17). About 23.08% isolates in genotype 3 expressed *tetO* while 30.77% isolates in genotype 3 expressed *tetA* genes. About 28.57% isolates in genotype 4 harboured *tetO* and *tetA* genes. Hence isolates from genotype 2 and genotype 4 are equally likely to be resistant to tetracycline due to efflux pump and ribosomal protection. However isolates from genotype 3 are more likely to be resistant due to efflux pump rather than ribosomal protection.

Genotypic distribution of fluoroquinolone resistance genes

The *gyrA* gene that conferred resistance to nalidixic acid or fluoroquinolone resistance was examined [30]. Present study found no *gyrA* gene positive isolates (not shown). Seven isolates (26.92%) had the *gyrB* genes that conferred the resistance of fluoroquinolone antibiotics and gave 382 bp gene products (Figure 18). Our study is a contrast to previous report which states that fewer isolates (4.17%) were found to have silent mutations in *gyrB* gene when compared to *gyrA* gene [30]. Genotype 4 contained the highest number of *gyrB* genes (42.86%) followed by genotype 2 (25%) and genotype 3 (23.08%) (Figure 17). Hence resistance to fluroquinolone in *Campylobacter* is more likely due to mutations in the gyrB subunit of DNA gyrase rather than the gyrA subunit.

Conclusion

Our study used bioinformatics aided approach to genotype and detect virulence and antibiotic resistance genes. Pulsed Field Gel Electrophoresis (PFGE) is considered as the gold standard for genotyping [34] and *in silico* data matched to that obtained in conventional method [35]. The summary of the results have been illustrated in Table 4. We concluded that there is variation in prevalence across the genotypes. Certain genotypes are more threatening than others. Our data isolates from genotype 1 and 5 are not pathogenic since it didn't harbor any of gastroenteritis toxin genes (*cdtA, cdtB, cdtC*), genes associated with Guillain–Barré syndrome (*wlaN, cgtB, waaC*) or genes associated with adherence and invasiveness (*iam, pldA, virB11, ciaB*). These genotypes did not have genes associated with fluoroquinolone and tetracycline resistance. The cytolethal distending toxin genes has a higher prevalence in genotype 3. Since cdtB alone can exert cytotoxicity, all isolates from genotype 3 are capable of causing gastroenteritis. Based on the difference in the prevalence of Guillain–Barré syndrome associated genes among the genotypes, isolates from genotype 3 are more likely to exert their pathology through the mimicry of GM1 rather than GM2 whereas the reverse is true for isolates from genotype 2 and 4. All isolates from genotype 3 would exhibit heptosyltransferase I activity and would modify the inner core of LOS. Our data also suggests that that invasion and adherence by isolates from genotype 2 are unlikely to be mediated by phospholipase A. The same can be stated invasion via virB11 invasion-associated marker for genotype 4. The mechanism of drug resistance also varies across the genotype. Isolates from genotype 2 and genotype 4 are equally likely to be resistant to tetracycline due to efflux pump and ribosomal protection. However isolates from genotype 3 are more likely to be resistant due to efflux pump rather than ribosomal protection. As for resistance to fluoroquinolone resistance isolates

from genotype 4 are the most resistant. All resistant isolates carry a mutated gyrB subunit of DNA gyrase rather than a mutated gyrA subunit. *Campylobacter* shows resistance to the clinically important antibiotics and this rising trend is a concern for public health. Therefore, enhanced research efforts are needed to understand the transmission, persistence and prevention of antibiotic-resistant *Campylobacter*. *Campylobacter* species acquired antibiotic resistance gene by horizontal gene transfer from either *Streptomyces*, *Streptococcus*, or *Enterococcus* species [59]. Seventy-five to seventy-six percent sequence homology was found between *tetO* gene of *Campylobacter* and *tetM* of *Streptococcus pneumoniae*. The virulence and resistance profile of future isolates with known genotype can be predicted according to our data. Additional studies are needed to understand how antibiotic resistant *Campylobacter* emerge under selective pressure. Application of advanced approaches, such as genomics and proteomics, is expected to provide new insights into the molecular mechanisms involved in the development of antibiotic resistance in *Campylobacter*. Good hygiene practice and food safety assurance programs should be implemented to reduce the contamination risk during handling and processing. Misuse of antibiotics in animal feed must be reduced to control antibiotic-resistant *Campylobacter*.

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