Virulence and Resistance Genes in *Campylobacter Spp* Isolated from Asymptomatic Children at Kibera Slum in Nairobi, Kenya

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Abstract

BACKGROUND

***Campylobacter spp.* are among the global causes of food poisoning and harbour virulence and resistance genes which play a role in invasion and antibiotic resistance. This study aimed to detect selected resistance and virulence genes in archived *Campylobacter spp*. isolated from children in Kibera, Nairobi, Kenya.**

MATERIALS AND METHODS

This was a retrospective laboratory-based cross-sectional study on archived samples of *Campylobacter spp.* at the Department of Public Health, Pharmacology and Toxicology, University of Nairobi. *Campylobacter* isolates were cultured on modified charcoal cefoperazone deoxycholate agar (mCCDA) media. The presence of selected virulence genes and resistance genes was detected by multiplex polymerase chain reaction (mPCR). STATA13 was used in data analysis. Binary logistic regression was used to ascertain the association between the genes and the *Campylobacter spp*.

RESULTS

The *Campylobacter spp.* under this study were *Campylobacter jejuni (C. jejuni*) (50%), *Campylobacter coli (C. coli)* (38%) and *Campylobacter lari (C. lari)* (12%). The virulence genes detected were *cgtB* 37 (25%), *pldA* (20%), *cdtA* (17%), *cdtB* (16%), *cdtC* (6%), *wlaN* (5%), *iam* (4%), *ciaB* (4%) and VirB*11* (3%). The virulence gene *waaC* was not detected in any of the isolates. The resistance genes detected were *TetA* (42%), *Tet B* (26%), *GyrB* (18%) and *GyrA* (14%).

CONCLUSION

The study revealed the presence of various virulence and resistance genes in *Campylobacter spp.* isolated from children. This information could help guide the development and enhancement of guidelines for the management of *Campylobacter* infections.

Keywords: *Campylobacter, Virulence Genes, Resistance Genes*

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Introduction

Campylobacter species infection is a bacterial infection that results in about 500 million cases of diarrhoea globally every year. The high incidence of diarrhoea and possible complications associated with *Campylobacter spp*. infections make it important from a socio-economic perspective (1).

Reports on the prevalence of *Campylobacter spp*. in humans in Sub-Saharan Africa show Nigeria being the highest at 62.7%, followed distantly by Malawi (21%) and South Africa (20.3%). For children below five years old, the prevalence of *Campylobacter* infections is 16.4% in Kenya, 15.5% in Rwanda and 14.5% in Ethiopia (2).

*Campylobacter*iosis is transmitted through contaminated water and food products, especially poultry. Upon exposure, symptoms develop within two to five days resulting in fever, stomach cramps and diarrhoea. *Campylobacter* infections are generally self-limiting. Severe cases may however necessitate treatment with ciprofloxacin, erythromycin, norfloxacin or azithromycin (2).

Campylobacter spp. has virulence and resistance genes which play a role during infection. Adhesion, invasion, colonization, and toxin production are expressed by virulence genes. The cytolethal distending toxin subunits (*cdtC, cdtA, and cdtB*) cause cellular distension leading to apoptosis. Other virulence genes including, *waaC, cgtB* and *wlaN* are lipo-oligosaccharides-associated genes, while *cgtB* and *wlaN* are involved in the production of B-1, 3 galactosyl transferase. The *iam* gene participates in different invasion steps (3).

Despite several studies indicating *Campylobacter* to be the cause of substantial human disease burden in low and middle-income countries, there is limited data on this bacteria's epidemiology in humans in Kenya. Elucidating the virulence and resistance genes in *Campylobacter spp.* would help in designing intervention strategies to curb and reduce disease progression. The outcome of this study will inform the development of better interventions to control drug resistance.

Materials and Methods

Study area

The archived bacteria isolates were obtained from faecal samples collected from the Kibera informal settlement in Nairobi, Kenya (4). The laboratory procedures were done at the Molecular laboratory, Kabete Campus, University of Nairobi.

Study design

This was a retrospective laboratory-based cross-sectional study done on archived isolates of *Campylobacter spp.* isolated from randomly collected 580 stool samples in July 2015. The stool samples were collected from school-going children of different ages and included both males and females attending 11 primary schools located in Kibera slums in Nairobi, Kenya. All the 106 previously identified and archived *Campylobacter* isolates collected in the "peepoo" sanitation project (4) were revived for this study.

Laboratory Procedures

Revival of archived *Campylobacter* isolates. The frozen bacterial isolates were revived by thawing at room temperature and sub-culturing on mCCDA media plates and incubated at the microaerophilic condition at 42˚C for 48 hours and examined for characteristic grey/white colonies with a moist appearance.

DNA extraction of *Campylobacter spp.*DNA extraction was done by suspending colonies from pure *Campylobacter spp.* in 0.5 mL of distilled water and heating in a water bath at 100 ˚C for 30 minutes. The preparation was cooled to room temperature and centrifuged at 15000 rpm. The supernatant was harvested and stored at -20 ˚C.

***Campylobacter species* identification.** *C. lari*, *C. jejuni* and *C. Coli* were verified by mPCR using specific gene primers. 25µl of the reaction mix was prepared by adding 12.5µl of PCR master-mix (Taq polymerase 0.05U/µl, 0.4mM of each dNTP and 4mM$MgCl_2$) (Biolabs Inc) to 10pmol of specific forward and reverse primers at 0.5 µL each and 2.5µl DNA template.

The PCR was done on a thermocycler (Applied Biosystems, model 9902, Singapore). Amplification was done using an optimized program set at 94˚ C for initial heating for 6 minutes. This was followed by thirty denaturation cycles at 95 ˚C for a minute. Annealing was done at 54 ˚C for one minute followed by an extension cycle at 70 ˚C for ten minutes. Storage of the PCR products was done at -20 ˚C while awaiting gel electrophoresis.

Visualization of the PCR products was done on 1.5% agarose gel (Genetics analysis grade, Fisher Scientific, New Jersey) prepared with 0.02% ethidium bromide stain. The amplicons were identified by matching to a molecular marker (100bp DNA ladder, England

Biolabs) that was run alongside the samples and the controls. The electrophoretic bands were captured using a UVP GelMax® 125 imager (Upland, CA, USA).

Detection of virulence and resistance genes in *Campylobacter spp.* A mPCR program was used to detect the presence of virulence genes (*ciaB, cdtC, cdtB, cdtA, pldA, virB11, iam, waaC, cgtB, and wlaN*) and resistance genes (*TetB, TeA, GyrB, GyrA*) using specific forward and reverse primers (Table 1) and DNA templates.

The mPCR was done on a thermocycler (Applied Biosystems) with similar procedures used in mPCR for *Campylobacter spp.* identification. Gene-specific primers and temperatures (Table 1) were however used for the annealing stage followed by an extension cycle at 70 ˚C for ten minutes. Storage of the PCR products was done at -20 ˚C. Visualization of the PCR products was done after gel electrophoresis on 1.5% agarose gel (Genetics analysis grade, Fisher Scientific, New Jersey).

Table 1:
Primers Used for Detection of Virulence and Resistance Genes in *Campylobacter spp.*

Genes		Primer sequence (5'-3')	Amplicon size (bp)	Annealing Temp (˚C)	References
Virulence Genes	*ciaB*	F: TGC GAG ATT TTT CGA GAA TG R: TGC CCG CCT TAG AAC TTA CA	527	56.5	(5)
	cdtC	F: TTGGCATTATAGAAAATA CAG TT R: CGATGAGTTAAAACAAAAAGATA	182	48	(6)
	cdtB	F: CAGAAAGCA AAT GGA GTG TT R: AGC TAA AAG CGG TGG AGT AT	620	51	(6)
	cdtA	F: CCT TGT GAT GCA AGC AAT C R: ACA CTC CAT TTG CTT TCT G	370	42	(3)
	pldA	F: AAG AGT GAG GCG AAA TTC CA R: GCA AGA TGG CAG GAT TAT CA	385	54	(5)
	iam	F: GCGCAAATATTATCACCC R: TTCACGACTACTACTATGCGG	518	55	(7)
	virB11	F: GAACAGGAAGTGGAAAAACTAGC R: TTCCGCATTGGGCTATATG	708	55	(8)
	waaC	F: TAATGAAAATAGCAATTGTTCGT R: GATACAAAAATCACTTTTATCGA	971	55.5	(9)
	wlaN	F: TGCTGGGTATACAAAGGTTGTG R: AATTTTGGATATGGGTGGGG	330	56	(10)
	cgtB	F: TTAAGAGCAAGATATGAAGGTG R: GCACATAGAGAACGCTACAA	562	56	(11)
Resistance genes	*TetB*	F: CCTTATCATGCCAGTCTTGC R: ACTGCCGTTTTTTCGCC	774	56	(12)
	TetA	F: GCTCACGTTGACGCAGGAAAG R: ATC GTC ATT GTC CGT TAC	486	60	(13)
	GyrB	F: ATGGCAGCTAGAGGAAGAGA R: GTGATCCATCAACATCCGCA	382	60	(14)
	GyrA	F: ACGCAAGAGATGGTT R: GCTGCGATGCGTTATACTGA	270	60	(14)

Data management and analysis

Data was recorded in Excel and STATA13 was used for analysis. A binary logistic regression reporting odds ratio was used to determine the association between the *Campylobacter* isolates and the detected genes. The test results were considered statistically significant at P≤0.05.

Ethical approval

Ethical approval was obtained from the Kenyatta University Ethical Clearance Committee and a license from National Commission for Science Technology and Innovation (NACOSTI), approval numbers (KU/ERC/COND.APPROVAL/VOL.1) and (NACOSTI/P/21/8228), respectively.

Results

Demographic characteristics of the *Campylobacter* species isolates

Sixty-six out of the one hundred and six archived *Campylobacter* bacteria isolates were successfully revived thus giving a revival rate of 62.3%. The demographic characteristics of the 66 revived isolates were distributed by age, gender, and village as shown in Table 2. Female participants were 34 (51.5%) while males 32 (48.5%). The majority were from participants aged >5 years. Silanga village accounted for most of the isolates 27 (40.9%). Out of the 66 isolates, 33 (50%) were *Campylobacter jejuni*, 25 (38%) *were Campylobacter coli* and 8 (12%) *were Campylobacter lari*.

Table 2:
Demographic Characteristics of *Campylobacter species* Isolated in Children from Kibera Slum

Variable		*C. coli*	*C. jejuni*	*C. lari*	Total
Age*	≤5 years	5(25%)	13(65%)	2(10%)	20
	>5 years	20(43%)	20(43%)	6(13%)	46
Gender	Male	11(34%)	17(53%)	4(13%)	32
	Female	14(41%)	16(47%)	4(12%)	34
Village	Gatwekera	1(20%)	4(80%)	0(0%)	5
	Laini Saba	2(11%)	12(67%)	4(22%)	18
	Lindi	8(53%)	6(40%)	1(7%)	15
	Mashimoni	1(100%)	0(0%)	0(0%)	1
	Silanga	13(48%)	11(41%)	3(11%)	27
Total		25(38%)	33(50%)	8(12%)	66

*Age stratification informed by vulnerability to infectious diseases in children below 5 years of age (15)

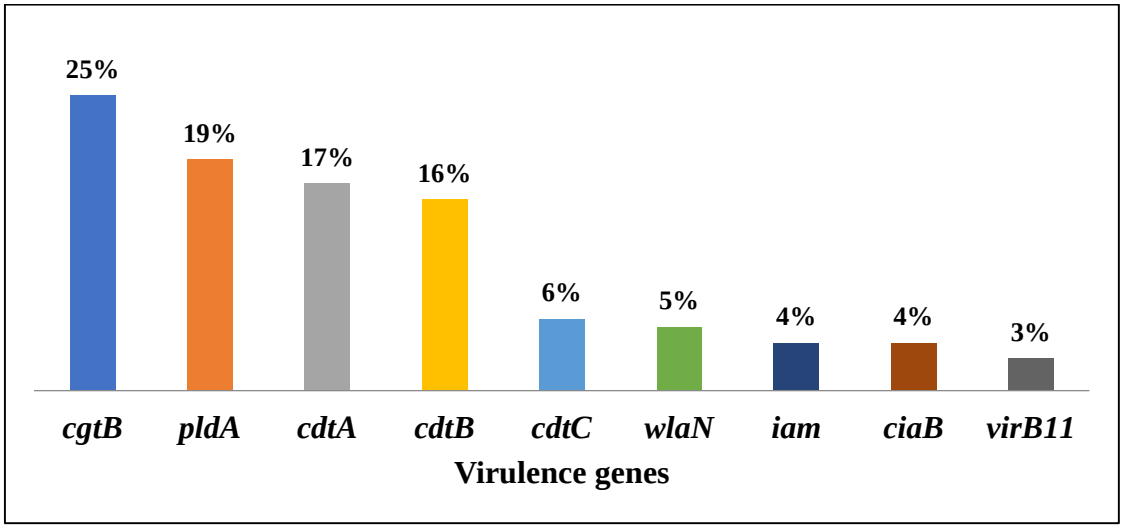

Figure 1:
Prevalence of virulence genes in *Campylobacter* isolates from children in Kibera slum

Prevalence of virulence genes in the *Campylobacter species* isolates

The virulence genes under study were *cdtA, cdtB, cdtC, cgtB, wlaN, waaC, virB11, pldA, ciaB* and *iam*. All these genes were detected in the study isolates except *waaC*. The most prevalent virulent gene in the isolates was *cgtB* 37 (25%) while *VirB11*4 (3%) was the least (Figure 1).

cgtB was the most prevalent gene detected in *Campylobacter coli* 16 (30%) and *C. jejuni* 18 (23%) while *pldA* was the most prevalent gene detected in *C. lari* 4 (22%). The *iam* gene was not detected in any of the *C. lari* isolates. The dominant virulent gene detected in the ≤ 5 years age category was *cgtB* and *pldA* each at 9 (21%). *cgtB* also dominated among the >5 years age category at 28 (26%). *ciaB* and *cdtB* genes were equally distributed across males and females (Table 3).

In the logistic regression analysis, *the cdtA* gene was 54% more likely to be detected across all the *Campylobacter* isolates. Additionally, *cdtA* was 57% more likely to be detected in *C. jejuni (OR = 1.57, P>0.05)* and 35% less likely in *C. coli* (*OR= 0.65, P>0.05*). There was no statistical association between *cdtA* and any of the isolated species (*OR = 1.54, P>0.05*). *cdtB* gene was 2.4 times more likely to be detected in *C. coli* (OR = 2.36, *P = 0.346*). *cdtB* was also 50% more likely to be detected in *C. jejuni* (*OR = 1.5, P = 0.651*). There was no statistical association between *cdtB* and isolated species (*OR = 0.99, p>0.05*).

cdtC gene was 60% more likely to be detected across all the *Campylobacter* species (*OR = 1.602, P>0.05*). *cdtC* gene was 56% more likely to occur in *C. jejuni* isolates (OR = 1.56, *P = 0.703*). There was no statistical association between *cdtC* and any isolated species (*P>0.05*). *cgtB* gene was 13% more likely to be detected in all the isolated *Campylobacter* species (*OR = 1.135, P = 0.724*). *cgtB* gene was three times more likely to occur in *C. coli* (*OR = 2.962, P = 0.196*) and two times more likely to be detected in *C. jejuni* (*OR = 2, P = 0.392*). There was however no significant association between *the cgtB* gene and any of the isolated species (*P>0.05*).

wlaN gene was 67% less likely to be detected in all the isolated *Campylobacter* species (*OR = 0.326, P = 0.039*). There was, however, a statistically significant association between this gene and the isolated species (*P<0.05*). There was also a significant statistical association between the *wlaN* gene and *C. jejuni* (*P<0.05*).

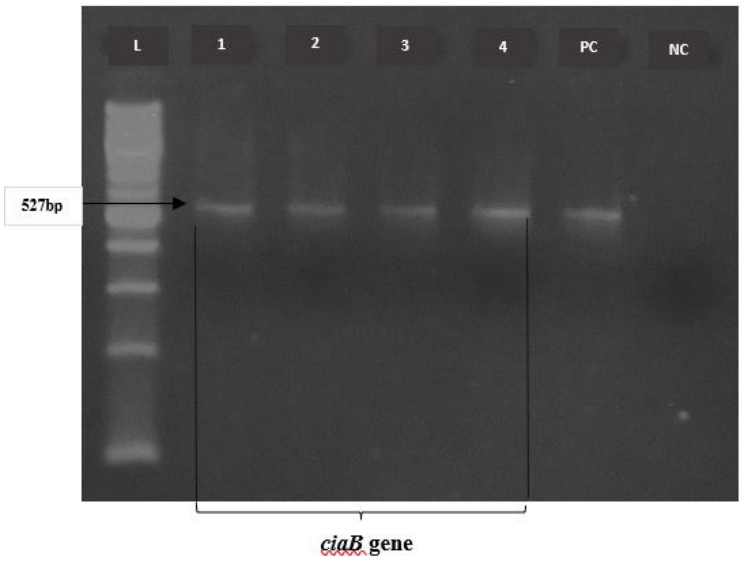

Figure 2:
Agarose Gel Electrophoresis Image *ciaB* Genes in *Campylobacter spp* isolates; lane 1-4 at 527bp, Followed by Positive Control and DNase. RNase Water as a Negative Control.

As for the least detected virulence gene *virB11* (3%), there was no statistically significant association between it and any of the species (*P>0.05*) (Table 3).

Prevalence of resistance genes in *Campylobacter* species isolated from children in Kibera slums

The resistance genes under study were *GyrA, GyrB, TetA* and *Tet B*. *TetA* was most prevalent at 36 (42%) while *GyrA* was the least detected at 12 (14%) (Figure 4).

TetA was dominant in *C. jejuni* 19 (53%) and least dominant in *C. lari* 3 (8%). *TetB* was dominant in *C. coli* 11 (50%) and least in *C. lari* (5%). *GyrA* was most detected in *C. jejuni* 6 (50%) and least detected in *C. lari* 1 (8%). *GyrB* was dominant in *C. jejuni* 9 (60%) and least dominant in *C. lari* 2 (13%). *TetA* had a higher distribution in males 21 (58%) compared to females 15 (42%). Silanga village had the highest number of resistance genes 15 (42%). *TetA* was the dominant resistance gene detected in Laini saba 11 (46%) and Silanga 36 (42%). *TetA* 1 (50%) and *TetB* 1 (50%) were the only resistance genes present in Mashimoni while *GyrA* and *GyrB* were notably absent in this village (Table 4).

In the logistic regression analysis, *the TetA* gene was 35% more likely to be detected in all *Campylobacter* species. There was however no significant association between *TetA* and the isolates (OR =1.354, *P > 0.05*). *TetA* was 2.1 times more likely to be detected in *C. coli* (OR = 2.121, *P = 0.367*) and 2.3 times more likely to be detected in *C. jejuni* (OR = 2.262, *P =0.314*). Tet B gene was 10% more likely to be detected in all the isolated species (OR = 1.101, *P = 0.801*). *TetB* gene was 5.5 times more likely to be detected in *C. coli* (OR = 5.5, *P = 0.136*) and 3 times more likely to be detected in *C. jejuni* (OR = 3.043, *P = 0.326*). There was however no significant association between any *Campylobacter* species and the *TetA* gene (*P>0.05*).

GyrA was 10% more likely to occur in the isolated *Campylobacter* species (OR = 1.103, *P = 0.834*). There was however no significant association between *the GyrA* gene and the *Campylobacter* species. *GyrA* was 75% more likely to be detected in *C. coli* (OR =1.75, *P = 0.635*) and 56% more likely to be detected in *C. jejuni* (OR = 1.556, *P = 0.703*). *Gyr B* was 28% more likely to be detected in the *Campylobacter* isolates but was not significantly associated with the isolates (OR = 1.280, *P = 0.576*). Furthermore, this gene was 13 % more likely to be detected in *C. jejuni* (OR = 1.125, *P = 0.896*) and 43% less likely in *C. coli* (OR = O.571, *P = 0.569*). The *GyrB* gene was however significantly associated with the female gender (OR=0.255, *P<0.05*) (Table 4).

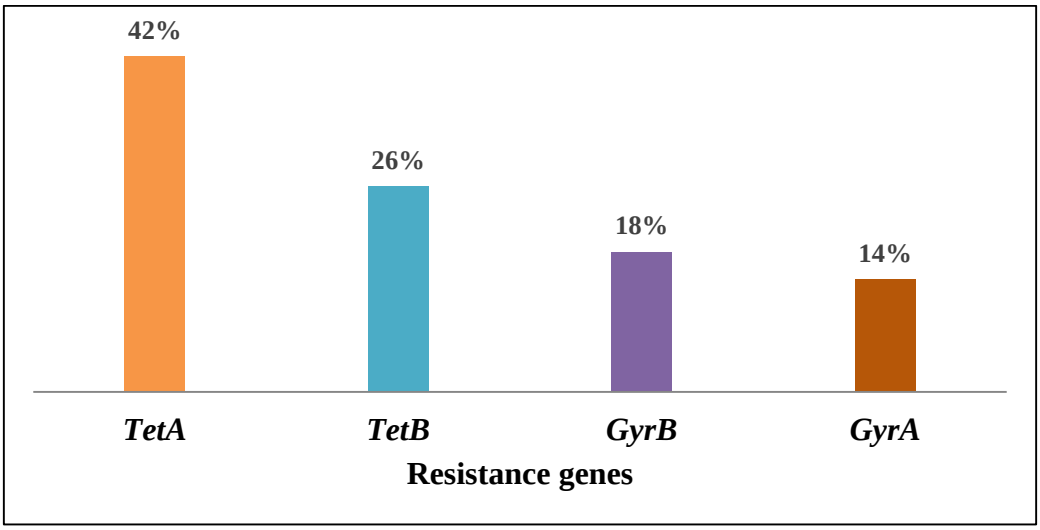

Figure 3:
Distribution of resistance genes in *Campylobacter spp.* isolated from children in the Kibera slum

Discussion

Although most studies on *Campylobacter* species in Kenya incline towards poultry and poultry products which are the main reservoirs of *Campylobacter* species, this study, however, focused on *Campylobacter* species from archived samples obtained from children. In this study selected virulence and resistance genes were detected in *Campylobacter spp* isolated from children in Kibera slums, Nairobi Kenya. This study thus documents the prevalence of the detected genes and their associations with the sociodemographic variables and *Campylobacter* isolates.

Most of the *Campylobacter* isolates identified in this study were *Campylobacter. jejuni* (50%). The dominance of *Campylobacter jejuni* could be due to its adaptability and virulence factors that enable it to survive in different hosts and the environment. This study finding agreed with previous studies that indicate *Campylobacter jejuni* as the dominant *Campylobacter species* in the environment (2,16).

Infections among children who dwell in low-income settings have been mainly attributed to poor sanitation (17). *Campylobacter* infections in this study were detected in both age categories with more infections reported in the age category >5 years 46 (69.7%). The higher prevalence of *Campylobacter* species in children above five years observed in this study could be due to the higher exposure of children in this age category to unhygienic conditions in their dwellings.

In this study, *Campylobacter* species harbored multiple virulence genes including *cgtB, pldA, cdtA, cdtB, cdtC, wlaN, iam, ciaB* and *virB11*. Other studies have also revealed these virulence genes to be present in various *Campylobacter* species (18,19). *waaC* gene was however not detected in this study (Figure 3). While *cgtB* was the most prevalent virulence gene detected in this study 37 (25%), its percentage in *C. jejuni* species analyzed in this study was higher in comparison to a study in Argentina which analyzed seven virulence genes with *cgtB* at 6.7% (17).

The *wlaN* gene helps in invasion and colonization by *C. jejuni* and plays a role in initiating Guillan-Barre Syndrome (20). The prevalence of *wlaN* was 5%. Despite its low prevalence, there was a statistically significant association between this gene and the *Campylobacter* isolates ($P < 0.05$). The prevalence of *the wlaN* gene in *C. jejuni* (2.6%) in this study was lower compared to a study done in Argentina which had the *wlaN* gene present in 20% of *C. jejuni* isolates (20). Other studies that have screened for virulence genes have failed to detect *wlaN* in some *Campylobacter* isolates (21). The least detected virulence gene, *virB11* had a prevalence of 3%. This was close to 3.6% obtained in a study done by (21). Similar findings of the low prevalence of *virB11* have also been noted in other studies (23–25).

The *Campylobacter* isolates in this study harboured resistance genes *GyrA, GyrB, TetB* and *TetA. The TetA* gene, which was the most prevalent in this study (Figure 2), codes for an efflux pump which causes high resistance to tetracycline (26). *TetA* gene dominated in all the *Campylobacter* isolates. These findings contradicted a study done on faeces and cloacal swabs of chicken in Kenya, where *the TetA* gene was in 90.3% of *C. jejuni* and all *C. lari* isolates (14). The high occurrence of the *TetA* gene could be attributed to continuous exposure of *Campylobacter* isolates to tetracycline, which is often readily available in retail pharmacies locally and a common antibiotic in poultry farming.

Conclusion

The increase in number of infections attributed to *Campylobacter* species locally remains a great concern to public health. This study determined selected virulence and resistance gene profiles of *Campylobacter spp* isolated from asymptomatic children at Kibera Slum in Nairobi, Kenya. By identifying the prevalent virulence genes in *Campylobacter spp*, this study provides information that may

help to explain *Campylobacter spp* transmission factors that could enhance susceptibility to infection among children. As for the prevalent resistance genes, the findings of this study provide more insight into the factors affecting antimicrobial resistance and inform on possible interventions and expected clinical outcomes during treatment of infections caused by *Campylobacter spp*.

Recommendations

There is still a constant need for up-to-date information that may help elucidate factors enhancing *Campylobacter* infections and their resistance to antimicrobial agents. Although this study showed the presence of selected virulence and resistance genes in local *Campylobacter* isolates, there is still a need for additional studies to screen for other virulence and resistance genes not tested for in this study.

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Conflict of Interest. The authors declared no conflict of interest.

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Appendix

Table 3:

Prevalence of Virulence Genes in *Campylobacter* Species Isolated from Children in Kibera Slums

Variable	Genes	*cdtA*	*CdtB*	*CdtC*	*CgtB*	*wlaN*	*virB11*	*pldA*	*ciaB*	*iam*	*waaC*	Total (N)
Species		*OR=1.54* CI:0.73-3.26 *P=0.255*	*OR=0.99* CI:0.48-2.04 *P=0.973*	*OR=1.602* CI:0.52-4.96 *P=0.414*	*OR=1.14* CI:0.56-2.29 *P=0.724*	*OR=0.33* CI:0.11-0.94 *P=0.039*	*OR=0.76* CI:0.19-3.08 *P=3.084*	*OR=1.00* CI:0.50-2.02 *P=0.996*	*OR=1.35* CI:0.37-4.93 *P=0.654*	*OR=2.23* CI:0.49-10.19 *P=0.299*	- - -	
Campylobacter spp	*C. coli*	7(13%) *OR=0.65* CI:0.12-3.47 *P=0.612*	11(21%) *OR=2.36* CI:0.40-14.04 *P=0.346*	2(4%) *OR=0.61* CI:0.05-7.76 *P=0.702*	16(30%) *OR=2.96* CI:0.57-15.40 *P=0.196*	3(6%) *OR=0.22* CI:0.04-1.48 *P=0.121*	1(2%) *OR=0.29* CI:0.02-5.28 *P=0.404*	10(19%) *OR=67* CI:0.14-3.30 *P=0.619*	1(2%) *OR=0.29* CI:0.02-5.28 *P=0.404*	2(4%) *OR=1.4E9* CI:0.0- *P=0.999*	0(0%)	53
	C. jejuni	16(20%) *OR=1.57* CI:0.32-7.66 *P=0.578*	11(14%) *OR=1.50* CI:0.26-8.69 *P=0.651*	6(8%) *OR=1.56* CI:0.16-15.12 *P=0.703*	18(23%) *OR=2.0* CI:0.41-9.78 *P=0.392*	2(2%) *OR=0.11* CI:0.01-0.81 *P=0.031*	2(2%) *OR=0.45* CI:0.04-5.71 *P=0.539*	15(19%) *OR=0.83* CI:0.18-3.91 *P=0.817*	4(5%) *OR=0.97* CI:0.09-10.04 *P=0.977*	4(5%) *OR=2.2E9* CI:0.0- *P=0.999*	0(0%)	78
	C. lari (R)	3(17%)	2(11%)	1(6%)	3(17%)	3(17%)	1(6%)	4(22%)	1(6%)	0(0%)	0(0%)	18
Age	≤5 years	7(17%) *OR=1.31* CI:0.44-3.89 *P=0.630*	6(14%) *OR=1.50* CI:0.49-4.62 *P=0.480*	5(12%) *OR=0.29* CI:0.07-1.21 *P=0.088*	9(21%) *OR=1.90* CI:0.66-5.50 *P=0.235*	1(2%) *OR=3.41* CI:0.39-29.74 *P=0.267*	1(2%) *OR=1.36* CI:0.13-13.58 *P=0.812*	9(21%) *OR=0.94* CI:0.33-2.70 *P=0.909*	1(2%) *OR=2.32* CI:0.25-21.23 *P=0.457*	3(7%) *OR=0.40* CI:0.07-2.16 *P=0.284*	0(0%)	42
	>5 years (R)	19(18%)	18(17%)	4(4%)	28(26%)	7(7%)	3(3%)	20(19%)	5(5%)	3(3%)	0(0%)	107
Gender	Female	12(17%) *OR=0.70* CI:0.26-1.89 *P=0.483*	12(17%) *OR=0.91* CI:0.33-2.48 *P=0.852*	6(9%) *OR=2.07* CI:0.47-9.10 *P=0.335*	16(23%) *OR=0.47* CI:0.17-1.26 *P=0.131*	1(1%) *OR=0.11* CI:0.01-0.94 *P=0.044*	3(4%) *OR=3.0* CI:0.30-30.44 *P=0.353*	15(21%) *OR=1.02* CI:0.38-2.69 *P=0.976*	3(4%) *OR=0.94* CI:0.18-5.01 *P=0.938*	2(3%) *OR=0.44* CI:0.07-2.57 *P=0.36*	0(0%)	70
	Male (R)	14(18%)	12(15%)	3(4%)	21(27%)	7(9%)	1(1%)	14(18%)	3(4%)	4(5%)	0(0%)	79

Table 3 (Cont'):

Residence (Village)	Gatwekera	1(10%) *OR=0.50* CI:0.05-5.15 *P=0.560*	1(10%) *OR=0.36* CI:0.04-3.71 *P=0.393*	1(10%) *OR=1.1* CI:0.10-12.09 *P=0.938*	3(30%) *OR=1.2* CI:0.17-8.38 *P=0.854*	0(0%) - - -	0(0%) - - -	3(30%) *OR=1.88* CI:0.27-13.09 *P=0.526*	0(0%) - - -	1(10%) *OR=3.13* CI:0.23-43.02 *P=0.394*	0(0%)	10
	Laini Saba	8(20%) *OR=1.6* CI:0.47-5.46 *P=0.453*	6(15%) *OR=0.73* CI:0.21-2.53 *P=0.616*	1(2%) *OR=O.26* CI:0.03-2.43 *P=0.237*	8(20%) *OR=0.64* CI:0.19-2.12 *P=0.466*	4(10%) *OR=1.64* CI:0.35-7.64 *P=0.527*	1(2%) *OR=0.47* CI:0.05-4.92 *P=0.529*	9(22%) *OR=1.25* CI:0.38-4.13 *P=0.715*	3(7%) *OR=2.5* CI:0.37-16.72 *P=0.345*	1(2%) *OR=0.74* CI:0.06-8.77 *P=0.808*	0(0%)	41
	Lindi	8(25%) *OR=2.29* CI:0.63-8.32 *P=0.210*	5(16%) *OR=0.73* CI:0.19-2.72 *P=0.636*	2(6%) *OR=0.68* CI:0.11-4.00 *P=0.667*	10(31%) *OR=1.6* CI:0.43-5.96 *P=0.484*	0(0%) - - -	0(0%) - - -	4(13%) *OR=0.46* CI:0.12-1.80 *P=0.260*	1(3%) *OR=0.89* CI:0.07-10.75 *P=0.929*	2(6%) *OR=1.92* CI:0.24-15.26 *P=0.536*	0(0%)	32
	Mashimoni	0(0%) - - -	1(33%) *OR=2.35E9* CI:0.0- *P=1.000*	0(0%) - - -	1(33%) *OR=1.29E9* CI:0.00- *P=1.000*	0(0%) - - -	0(0%)	1(33%) *OR=2.02E9* CI:0.00- *P=1.000*	0(0%) - - -	0(0%) - - -	0(0%)	3
	Silanga (R)	9(14%)	11(17%)	5(8%)	15(24%)	4(6%)	3(5%)	12(10%)	2(3%)	2(3%)	0(0%)	63
Total (n)		26 (17%)	24(16%)	9(6%)	37(25%)	8(5%)	4(3%)	29(19%)	6(4%)	6(4%)	0(0%)	149

Total (n) represents the total number of a specific gene in all the isolates while Total (N) represents the total number of all genes in a specific variable.
Percentage $(\%) = \frac{\mathbf{n}}{\mathbf{N}} \times \mathbf{100}$**,** OR**=Odds Ratio,** CI**=95% Confidence Interval,** R**= Reference group in OR analysis**

Table 4: Prevalence of resistance genes in *Campylobacter* species isolated from children in Kibera slums

Variable	Genes	*TetA*	*TetB*	*GyrA*	*GyrB*	Total (N)
	Species	OR=1.354, CI:0.669-2.741 *P=0.400*	OR=1.101, CI:0.522-2.323 *P=0.801*	OR=1.103, CI:0.440-2.767 *P=0.834*	OR=1.280, CI:0.538-3.042 *P=0.576*	
Campylobacter spp.	*C. coli*	14(39%) OR=2.121, CI:0.414-10.878 *P=0.367*	11(50%) OR=5.5, CI:0.586-51.620 *P=0.136*	5(42%) OR =1.75, CI:0.173-17.686 *P = 0.635*	4(27%) OR = O.571, CI:0.083-3.916 *P = 0.569*	34
	C. jejuni	19(53%) OR=2.62, CI:0.462-11.082 *P=0.314*	10(45%) OR=3.04, CI:0.330-28.104 *P=0.326*	6(50%) OR =1.556, CI:0.160-15.123 *P = 0.703*	9(60%) OR = 1.125, CI:0.191-6.633 *P = 0.896*	44
	C. lari (R)	3(8%)	1(5%)	1(8%)	2(13%)	7
Age	≤5 years	13(36%) OR=0.538, CI:0.182-1.594 P = 0.264	6(27%) OR=1.244, CI:0.401-3.862 P = 0.705	4(33%) OR = 0.842, CI:0.222- 3.199 P = 0.801	5(33%) OR = 0.833, CI:0.243-2.854 P = 0.772	28
	>5 years (R)	23(64%)	16(73%)	8(67%)	10(67%)	57
Gender	Female	15(42%) OR=0.414, CI:0.153-1.119 P=0.082	9(41%) OR=0.526, CI:0.186-1.486 P=0.225	4(33%) OR=0.4, CI:0.107-1.490 P=0.172	4(27%) OR=0.255, CI:0.071-0.909 P=0.035	32
	Male (R)	21(58%)	13(59%)	8(67%)	11(73%)	53
Residence (Village)	Gatweker A	2(6%) OR=0.533, CI:0.076-3.725 *P=0.526*	1(5%) OR=0.425, CI:0.042-4.352 *P=0.471*	1(8%) OR=1.438, CI:0.126-16.410 *P=0.770*	0(0%) - -	4
	Laini Saba	11(31%) OR=1.257, CI:0.373-4.233 *P=0.712*	4(18%) OR=0.486, CI:0.125-1.889 *P=0.297*	4(33%) OR=1.643, CI:0.353-7.640 *P=0.527*	5(33%) OR=1.099, CI:0.287-4.211 *P=0.891*	24
	Lindi	7(19%) OR=0.7, CI:0.197-2.485 *P=0.581*	6(27%) OR=1.133, CI:0.310-4.138 *P=0.850*	3(25%) OR=1.438, CI:0.276-7.497 *P=0.667*	3(20%) OR=0.714, CI:0.155-3.299 *P=0.666*	19
	Mashimoni	1(3%) OR=1.29E9, CI:0.0 - *P=1.000*	1(5%) OR=2.75E9, CI:0.0- *P=1.000*	0(0%) - -	0(0%) - -	2
	Silanga (R)	15(42%)	10(45%)	4(33%)	7(47%)	36
Total (n)		36 (35%)	22(29%)	12(16%)	15(20%)	85

Total (n) represents the total number of a specific gene in all the isolates while Total (N) represents the total number of all genes in a specific variable

Percentage $(\%) = \frac{\mathbf{n}}{\mathbf{N}} \times \mathbf{100}$**,** OR**=Odds Ratio,** CI**=95% Confidence Interval,** R**= Reference group in OR analysis.**