



RESEARCH ARTICLE

REVISED Multidrug-resistant *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter lari* isolated from asymptomatic school-going children in Kibera slum, Kenya [version 2; peer review: 1 approved, 2 approved with reservations]

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v2 First published: 07 Feb 2020, 9:92
<https://doi.org/10.12688/f1000research.21299.1>
 Latest published: 04 Sep 2020, 9:92
<https://doi.org/10.12688/f1000research.21299.2>

Abstract

Background: The objective of this study was to determine the prevalence of thermophilic *Campylobacter* spp. in asymptomatic school-going children and establish the antibiotic resistance patterns of the isolates towards the drugs used to treat campylobacteriosis, including macrolides, quinolones and tetracycline. *Campylobacter* spp. are a leading cause of enteric illness and have only recently shown resistance to antibiotics.






Methods: This study isolated *Campylobacter* spp., including *Campylobacter coli*, *Campylobacter jejuni* and *Campylobacter lari*, in stool samples from asymptomatic school-going children in one of the biggest urban slums in Kenya. The disc diffusion method using EUCAST breakpoints was used to identify antibiotic-resistant isolates, which were further tested for genes encoding for tetracycline resistance using primer-specific polymerase chain reaction.

Results: In total, 580 stool samples were collected from 11 primary schools considering both gender and age. Subjecting 294 biochemically characterized *Campylobacter* spp. isolates to genus-specific PCR, 106 (18.27% of stool samples) isolates were confirmed *Campylobacter* spp. Out of the 106 isolates, 28 (4.83%) were *Campylobacter coli*, 44 (7.58%) were *Campylobacter jejuni* while 11 (1.89%) were *Campylobacter lari*. *Campylobacter jejuni* had the highest number of isolates that were multi-drug resistant, with 26 out of the 28 tested isolates being resistant to ciprofloxacin (5 mg), nalidixic acid (30 mg), tetracycline (30 mg) and erythromycin (15 mg).

Conclusions: In conclusion, asymptomatic school going children in the study area were found to be carriers of multidrug resistant

Open Peer Review

Approval Status   

	1	2	3
version 2			
(revision)			
04 Sep 2020	view		view
			
version 1			
07 Feb 2020	view	view	

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Any reports and responses or comments on the article can be found at the end of the article.

Campylobacter coli, *Campylobacter jejuni* and *Campylobacter lari* at 84%. A one-health approach, which considers overlaps in environment, animals and human ecosystems, is recommended in addressing multidrug resistance in *Campylobacter*, since animals are the main reservoirs and environmental contamination is evident.

Keywords

Multidrug, resistance, *Campylobacter*, genes, asymptomatic



This article is included in the **Pathogens** gateway.



This article is included in the **Antimicrobial Resistance** collection.

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Competing interests: No competing interests were disclosed.

Grant information: This research received funding from Grand Challenges Canada (grant number S7 0659-01-10) and Swedish Research Council VR (reference No.348-2014-3508).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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How to cite this article: Gitahi N, Gathura PB, Gicheru MM *et al.* **Multidrug-resistant *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter lari* isolated from asymptomatic school-going children in Kibera slum, Kenya [version 2; peer review: 1 approved, 2 approved with reservations]** F1000Research 2020, 9:92 <https://doi.org/10.12688/f1000research.21299.2>

First published: 07 Feb 2020, 9:92 <https://doi.org/10.12688/f1000research.21299.1>

REVISED Amendments from Version 1

A part from typing errors and grammar, the major difference is in conclusions, I have articulated my conclusions derived from this study and lined my recommendations to this study.

Any further responses from the reviewers can be found at the end of the article

Introduction

Campylobacter spp. infection is a leading cause of enteric illness^{1,2}, manifesting as mild-to-severe diarrhoea with watery loose stool that is often followed by bloody diarrhoea³. Infections also manifest as meningitis, pneumonia, miscarriage, severe form of Guillain-Barre syndrome (GBS) and reactive arthritis (ReA) and irritable bowel syndrome (IBS)³⁻⁵. Isolation of pathogenic *Campylobacter* spp. from asymptomatic children would be as a result of the pathogens not expressing the virulence factor cytolethal distending toxin, which is able to induce host cell apoptosis⁶. Pathogenesis could also be influenced by host immune system and pathogens adaptation strategies⁶. Other factors like motility and chemotaxis affect effective *Campylobacter* colonization and pathogenesis; these have been shown to vary in mutants⁷.

Campylobacter spp. are found in the intestinal tract of wild and domestic animals, particularly in birds, asymptotically as temporal carriers but causing illness in humans³. The bacteria can survive up to five months at -20°C but die off in a few days at room temperature^{5,8,9}. *Campylobacter* spp is vulnerable to air exposure, drying, low pH and heating³. Three species, namely *C. jejuni*, *C. coli* and *C. lari*, account for 99% of human *Campylobacter* spp. isolates, with *C. jejuni* accounting for 90% of the isolates. *C. fetus* and *C. upsaliensis* have also been isolated in humans¹⁰⁻¹².

Distinguishing between *Campylobacter* species using phenotypic methods is difficult; however, genotypic methods have been developed that are capable of differentiating the species. This has enabled more elaborate epidemiological understanding of Campylobacteriosis, identification of the sources and routes of infection^{13,14}. The use of multiplex PCR methods has resulted in cheap, rapid and sensitive genetic identification of *Campylobacter* spp¹⁵.

It was not until the last two decades that *Campylobacter* spp. was shown to exhibit multidrug resistance (MDR). Before that, the bacteria were considered to be susceptible¹⁶. Tetracycline is one of the antimicrobial agents against which *Campylobacter* spp. have shown resistance. In *Campylobacter* spp., tetracycline resistance has been reported to be mediated by more than one tetracycline resistance (*tet*) genes. The *tet(O)* and *tet(S)* genes are the ribosomal protection protein and plays the primary part in tetracycline resistance in *C. jejuni* and *C. coli*^{17,18}. This is transferred as a plasmid encoded gene¹⁹ or as non-self-mobile form. The *tet(A)* gene encodes the 46 kDa membrane-bound efflux protein. This protein carries tetracycline from the cell membrane and its first known resistance role in *Campylobacter* spp. was reported in 2014¹⁶.

In *Campylobacter* spp. resistance to quinolones is mainly due to a single point mutation in the quinolone resistance determination region of *gyrA* gene (QRDR)^{20,21}, at amino acid 86 by replacement of Thr by Ile²². Occasionally, mutation in topoisomerase IV (ParC) results to resistance against quinolones. Other amino acids substitutions have been reported by Piddock *et al.* and others²³⁻²⁶. In *Campylobacter* there has been no documented mutational change to the *gyrB* subunit gene in relation to resistance against quinolones; however, Piddock *et al.*²², and Changkwanyun *et al.*²⁷ noted that resistance to ciprofloxacin in *Campylobacter* is mediated by mutations on the *gyrA* gene.

Methods

Study area and background

The study was carried out at primary schools located at Kibera informal settlement, Nairobi County, Kenya in July 2015. Kibera is located at an altitude of 1670 m above sea level, at latitude 36°50' east and longitude 1°17' south, about 140 km south of the equator. Kibera is located 5 km South of Nairobi Central Business District (CBD), the Capital of Kenya. Kibera is divided into 9 official villages. The average living place is 3 m², with an average of 5 persons per place. The study site presents a population with diverse enteric infections²⁸. In total, 11 primary schools with pupil population ranging from 120 to 189 were randomly sampled and, 40 to 80 stool samples collected from pupils in each school, depending on the school population, making a total of 580 stool samples. With a known prevalence of 40.7% of soil transmitted helminths in school going children in urban Kenya, the formula by Martin *et al.* (1998) was used to determine the desired minimum sample size. The schools were distributed in five administrative villages, namely Lindi, Silanga, Laini Saba, Gatwekera and Mashimoni. Participants' parents provided written consent through the care givers. This was done during parents' school meetings, where parents were informed of the intended study and its benefits, those who agreed their children to participate were issued with consent forms for them to sign and return to their class teacher. Only those who their parents consented participated in the study.

Research clearances were given by National Commission for Science, Technology and Innovation (research clearance permit No. 3756) and ethical clearance (PKU/278/1274) was granted by Kenyatta University Ethical Review Committees.

***Campylobacter* spp. culture.** In the laboratory, 5 g of freshly collected faecal sample was pre-enriched by suspending the faeces in 45 ml buffered peptone water (BPW) (Oxoid, Hampshire, England) and incubating the suspension at 42°C for 18 hours in a 50-ml closed culture tube. The pre-enrichment was inoculated onto modified campylobacter charcoal-cefoperazone deoxycholate (mCCDA) agar plates with supplement (polymyxin B 2500IU, rifampicin 5 mg, trimethoprim 5 mg and cycloheximide 50 mg) using a sterile swab and the plates incubated at 45°C for up to 48 hours under anaerobic conditions. The mCCDA culture media (Oxoid, Hampshire, England) was prepared according to manufacturer's instructions and stored at 4°C until use. Micro-aerobic conditions were achieved by adding a 21.3-g sachet of CampyGen™ 3.5 L (Oxoid, Hampshire, England) in an anaerobic jar with the cultures resulting to a

maximum of 13.2% O₂ within 24 hours and 9.5% CO₂ in 1 hours. After 24 hours of incubation, the plates were checked for characteristic growth and plates without growth were re-incubated for an additional 24 hours. Characteristic colonies (grey/white or creamy grey in colour with moist appearance) were examined and counted. Distinct colonies were harvested and tested for oxidase and peroxidase breakdown, by picking a portion of distinct colony with a sterile wire loop and placing it on a drop of 30% hydrogen peroxide on a clean microscope slide. Production of effervescent air bubbles was recorded as peroxidases positive. The same colonies were tested for cytochrome oxidase enzyme production by placing a portion of the test colony onto oxidase paper impregnated with NNN'N' tetramethyl-p-phenylene-diamine dihydrochloride (Oxoid, Basingstoke, UK). Purple colour change was recorded as positive reaction. Reactive colonies were processed for DNA and a portion stored in skimmed milk at -80°C for further characterization.

DNA preparation from bacteria colonies and multiplex PCR.

For DNA extraction, three distinct colonies from pure bacteria cultures were picked with a sterile wire loop and suspended in 0.5 ml sterile, distilled water. The suspension was boiled for 30 minutes in a water bath. After cooling to room temperature, the preparation was centrifuged at 2000 x g and the supernatant harvested and stored at -20°C until analysis by polymerase chain reaction (PCR). PCR was first undertaken to confirm *Campylobacter* genus for the isolates after which three specific species were also identified: *C. coli*, *C. jejuni* and *C. lari*. The *Campylobacter* DNA preparation (2 µl) was amplified in a 25 µl reaction mix by mixing 2.5 µl 10X PCR buffer (Coraload), 0.5 µl dNTPs, 0.125 µl Taq DNA polymerase (Inqaba biotec, Pretoria, South Africa) and 0.1 µl of each specific primer to 10 pmole (Inqaba Biotec, Pretoria, South Africa), 2 µl DNA template and 18.657 µl DNase/RNase-free distilled water. The DNA was amplified using a program of initial heating at 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 1 minutes, annealing at 56°C for 1 min, extension at 72°C for 1 min with a final extension of 72°C for 10 min using a Veriti 96 wells thermocycler, (Applied Biosystems, model 9902, Singapore) in 0.2-ml PCR tubes. The PCR products were kept at -20°C until gel electrophoresis was done.

The *Campylobacter* genus-specific primers, C412F and C1228 R, described by Linton *et al.*²⁹ were used to amplify a 812 bp fragment within the 16S rRNA gene of *Campylobacter* species using forward primer C412F 5'-GGATGACACTT TTCGAGC-3' and reverse primer; C1228R 5'-R-CATTGTAGC ACGTGTGTC-3'. Multiplex PCR was carried out for *C. jejuni* and *C. coli* with specific primers CjejlpxAF, CjejipxAR (shared by both species) and CcollpxAF, described by Klena *et al.*¹⁵ to amplify 331 bp and 391 bp fragment flanking the *lpxA* gene. The primer sequences were; CjejlpxAF (forward) 5'-ACAACCTTGGTGACGATGTTGTA-3', CjejipxAR (reverse, shared by CjejlpxA and CcollpxA) 5'-CAATCATGDGCDATATGASAATAHGCCAT-3' for *C. jejuni* and for *C. coli* CcollpxAF (forward) 5'-AGACAATAAGAGAGAATCAG -3'. The *C. lari* specific primers were forward primer lpxAC, 5'-AGACAATAAGAGAGAATCAG-3' and reverse primer lpx-ARKK2M, 5'-CAATCATGDGCDATATGASAATAHGCCAT-3'.

The PCR products were visualized by electrophoresis in a 1.5% agarose (Genetics analysis grade, Fisher Scientific, New Jersey) gel stained with 0.02% ethidium bromide and amplicons identified against molecular marker (50 bp DNA ladder, England Biolab) run alongside the samples.

For confirmation, the positively identified PCR products were submitted for sequencing. The PCR products were first purified using exonuclease1, shrimp alkaline phosphatase mixture (ExoSAP mix) according to the manufacturer's instructions. Briefly, this was done by adding 2.5 µl of ExoSAP mix to 10 µl PCR product. The mixture was then incubated at 37°C for 30 minutes and reaction stopped by heating at 95°C for 5 minutes. The clean PCR product was then quantified using a fluorimeter (Qubit 2.0, Invitrogen, USA). The clean DNA was first labelled with BigDye terminator v3.1kit (Applied Biosystem, CA, USA) according to the manufacturer's instructions and loaded into Genetic Analyzer (ABI 3730 capillary analyser; Applied Biosystems, Foster City, CA, USA) for sequencing. Sequences were obtained in ABI files that were opened and edited to remove unspecific ends using BioEdit version 7.0.4 (Hall, CA, USA) software. Clean sequences were then submitted to NCBI GenBank database and BLASTn program used to test for homology and genetic identity of bacteria isolates.

Antimicrobial sensitivity test (AST) for PCR-confirmed *Campylobacter* spp..

Campylobacter spp. isolates were phenotypically tested for resistance using selected antimicrobial agents according to European committee on antimicrobial susceptibility testing (EUCAST)³⁰. Only antibiotics with EUCAST established breakpoints were tested, namely tetracyclines (tetracycline 30 mg), quinolones (ciprofloxacin 5 mg, naladixic acid 30 mg) and macrolides (erythromycin 15mg). Mueller-Hinton agar plates plus 5% de-fibrinated horse blood with 20 mg/L β-nicotinamide adenine dinucleotide Mueller-Hinton fastidious (β-NAD (MH-F)); (Oxoid, Basingstoke, UK) were prepared and dried at 35°C, with the lid removed, for 15 min prior to inoculation to reduce swarming. Inoculum turbidity was adjusted to McFarland 0.5 prior to inoculation. The antibiotic discs were placed on the inoculated plates using a sterile multi-disc dispenser and incubated in a microaerobic environment at 41±1°C for 24 hours. Isolates with insufficient growth after 24 hours of incubation were re-incubated immediately and inhibition zones read after a total of 40–48 hours incubation. The inhibition zones were defined by the point showing no growth when viewed from the front of the plate with the lid removed and with reflected light.

Genotypic characterization of *Campylobacter* spp. isolates for antimicrobial resistance.

A total of 90 antibiotic resistant, *Campylobacter* spp. isolates including; 11 *C. lari*, 30 *C. coli* and 49 *C. jejuni* were selected and characterized with PCR for demonstration of genes encoding resistance to tetracyclines including *tet(A)*, *tet(B)*, *tet(C)* and *tet(O)*. Multiplex PCR was carried out as described above. Primers used for amplification of products encoding for the resistant genes to tetracyclines are shown in Table 1.

Table 1. Primers used for identifying tetracyclines encoding genes in selected bacteria isolates.

Primer sequence 5'-3'	Direction	PCR product, bp	genes	Reference
GTGAAACCCAACATACCCC	Forward	577	<i>Tet(A)</i>	31
GAAGGCAAGCAGGATGTAG	Reverse			
CCTCAGCTTCTCAACGCGT	Forward	635	<i>Tet(B)</i>	31
GCACCTTGCTGAGACTCTT	Reverse			
ACTTGAGCCACTATCGAC	Forward	880	<i>Tet(C)</i>	32
CTACAATCCATGCCAACCC	Reverse			
AACTTAGGCATTCTGGCTCAC	Forward	515	<i>Tet(O)</i>	33
TCCCACTGTTCCATATCGTCA	Reverse			

Results

Of the 580 stool samples collected in 11 schools in Kibera, 294 (51%) were phenotypically characterized as suspect *Campylobacter* spp. When these isolates were subjected to PCR using genus and species-specific primers, 106 (18%) isolates were confirmed to be *Campylobacter* spp. Among the 106 isolates, 28 (4.8%) were *C. coli*, 44 (7.6%) *C. jejuni* (Figure 1) while 11 (1.9%) were *C. lari*. In total, 23 (4.0%) *Campylobacter* isolates were not species identified as belonging to either *C. coli*, *C. jejuni* or *C. lari* (Table 2).

Antimicrobial sensitivity test (AST) for confirmed *Campylobacter* spp.

The EUCAST disk diffusion method was used to determine the resistance patterns of only the identified isolates, 68 (*C. jejuni*, *C. coli* and *C. lari*) confirmed by PCR (Table 2). Fifteen isolates were not recovered from storage culture after identification and thus not tested. All of the antibiotics studied had isolates showing resistance towards them, with 96% of isolates resistant to tetracycline (30 mg), 93% to naladixic acid (30 mg) and all the isolates tested resistant to erythromycin (15 mg). The antibiotic that most isolates were sensitive to was ciprofloxacin (5 mg) which still had 84% of the isolates showing resistance (Table 3). Of the four *tet* genes tested, *tet(A)* was most frequently identified in 20 (29.1%) of the isolates followed by *tet(O)* in 8 (11.7%) isolates and *tet(C)* in only 2 (2.9%) isolates. None of the isolates had more than one *tet* gene demonstrated. (Table 3).

Multidrug resistant profiles in *Campylobacter* spp. isolates

Four MDR profiles were observed. All of the tested isolates were resistant to two or more antimicrobial agents, but the majority of isolates (84%) were resistant to all the antibiotics studied (profile 3 and 4). *Campylobacter jejuni* had the highest number of isolates that were MDR with 25 (37%) isolates being resistant to all four antibiotics tested (profile 1). *C. coli* had 23 (34%) isolates resistant to all the antibiotics while *C. lari* had 9 (13%) isolates resistant to the four antibiotics. One (1.5%) *C. jejuni* and *C. lari* isolates was resistant

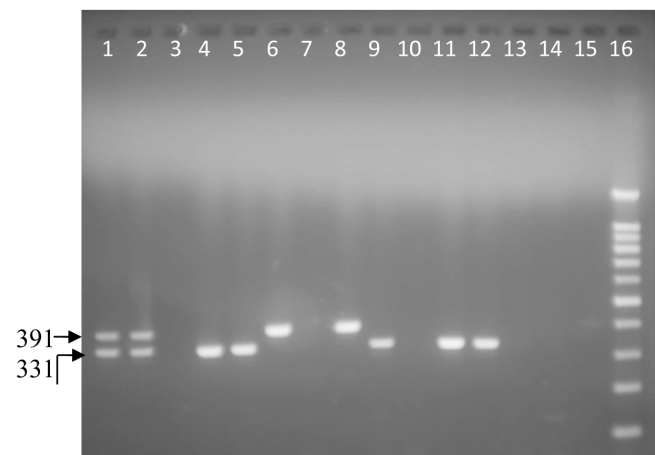


Figure 1. Ethidium bromide stained 1.5% agarose gel electrophoresis of *Campylobacter coli* (391 bp) and *C. jejuni* (331 bp) in a multiplex PCR with a 100-bp ladder. From left to right, lane 1 and 2 positive samples; mixture of *Campylobacter jejuni* and *Campylobacter coli* obtained from sequenced laboratory isolates (PHPT 1 & 2). Lane 3 negative control: purified water. Lanes 4, 5, 9, 11 and 12: *C. jejuni*. Lanes 6, 8 and 15: *Campylobacter coli*. Lanes 7, 10, 13 and 14: negative samples. Lane 16: 100-bp ladder.

to drugs in profile 2, while three (3%) *C. coli* isolates were in this profile. However, profile 4 had only one (1%) *C. coli* isolate while profile 4 had 2 (3%) *C. lari*, 4 (6%) *C. coli* and 5 (7%) *C. jejuni* MDR isolates (Table 4).

Discussions

A prevalence of 18% *Campylobacter* spp. in asymptomatic school going children was confirmed in this study. *Campylobacter* isolation from healthy children has been reported in developing countries³⁴ at a prevalence of 15%, which closely agrees with this study's findings. The authors attributed the infections with *Campylobacter* to close contact with reservoir animals like chickens, as well as poor sanitation²⁰. Both of these factors are prominent in this study area, where chicken share housing with humans. The isolates were further characterized

Table 2. Molecular characterization by polymerase chain reaction of *Campylobacter* spp. isolates from school going children's stool samples.

School	<i>C. coli</i>	<i>C. jejuni</i>	<i>C. lari</i>	Other <i>C. spp.</i>	Total <i>Campylobacter</i> spp.
A	0	0	0	5	8.5% (5/59)
B	3	0	2	3	21% (8/38)
C	0	0	2	3	8.1% (5/62)
D	6	7	0	2	34% (15/44)
E	8	9	1	2	25% (20/79)
F	0	3	0	1	21% (4/19)
G	5	10	1	2	23% (18/80)
H	0	3	1	1	9.4% (5/53)
I	0	4	1	0	17% (5/30)
J	5	6	2	2	22% (15/69)
K	1	2	1	2	13% (6/47)
Total	28	44	11	23	106
Prevalence	4.8% (28/580)	7.6% (44/580)	1.9% (11/580)	3.9% (23/580)	18.3% (106/580)

Table 3. Drug resistance patterns of pathogenic *Campylobacter* spp. isolates from school children's stool samples, n=68 using EUCAST disk diffusion method (2016) and presence of genes encoding tetracycline resistance.

Antimicrobial agent	Resistance genes (no. of isolates)	Resistant isolates (EUCAST, 2016)			
		<i>C. jejuni</i> (n=30)	<i>C. coli</i> (n=27)	<i>C. lari</i> (n=11)	Total Resistance (%)
Tetracycline (30 mg)	<i>Tet</i> (A) (20), <i>tet</i> (B) (0), <i>tet</i> (C) (2), <i>tet</i> (O) (8)	30	26	11	67 (96)
Ciprofloxacin (5 mg)	Genotyping not done	25	23	9	57 (84)
Naladixic acid (30 mg)		29	24	10	63 (93)
Erythromycin (15 mg)	Genotyping not done	30	27	11	68 (100)

Table 4. Multidrug resistance (MDR) *Campylobacter* spp. isolates profile by antimicrobial sensitivity testing.

Drug (dose) profiles	No of MDR resistant isolates per species			MDR <i>Campylobacter</i> spp. isolates (n=68)
	<i>C. jejuni</i> (n=30)	<i>C. coli</i> (n=27)	<i>C. lari</i> (n=11)	
1. Ciprofloxacin (5 mg), nalidixic acid (30 mg), tetracycline (30 mg), erythromycin (15mg)	25	23	9	57 (84%)
2. Nalidixic acid (30 mg), tetracycline (30 mg), erythromycin (15 mg)	1	3	1	5 (7.3%)
3. Ciprofloxacin (5 mg), erythromycin (15mg)	5	4	2	11 (16%)
4. Tetracycline (30 mg), erythromycin (15 mg)	0	1	0	1 (1.5%)

and *C. jejuni* was isolated more frequently (7.6%) as compared to *C. coli* (4.8%) and *C. lari* (2%), whereas 4% were none of the three species analysed. This distribution between *Campylobacter* species agrees with other reports from both developed and developing countries, including Kenya^{34–36}. Among the thermophilic *Campylobacter* species, *C. upsaliensis* was not characterised using PCR in this study.

The *Campylobacter* spp. resistant to tetracycline had more *tet*(A) genes than *tet*(O) genes which were found in 20 (29%) and 8(12%) isolates respectively. This is consistent with Nguyen *et al.*²⁰ who identified more *tet*(A) genes than *tet*(O) genes in Kenyan *Campylobacter* spp. isolates from chickens, at 35% and 13% respectively. The high resistance rates obtained in this study, with 84% of isolates being resistant to all four agents, was in agreement with the findings of Nguyen *et al.*²⁰ and Coker *et al.*³⁴ for chicken and human *Campylobacter* isolates, respectively. Both studies reported more than 70% resistance to ciprofloxacin, nalidixic acid and tetracycline. However, these results contrast with those on human *Campylobacter* from diarrhoea cases in Western Kenya, where resistance to ciprofloxacin were observed in 6% cases, to nalidixic acid in 26%, and to tetracycline in 18%. Erythromycin resistance in this study was also high, in contrast to the findings of Nguyen *et al.*²⁰ in chicken-isolated *Campylobacter*. In the setting of the current study, with domestic animals hosted within the human settlements and poor sanitation, the possibility of cross-infection is very likely, as is horizontal transfer of antimicrobial resistance-encoding genes. Ciprofloxacin and erythromycin are the drugs of choice for *Campylobacter* treatment. These drugs are often used in Kenya for self-treatment of infections other than gastroenteritis, and resistance can be expected to increase in developing countries³⁴.

In conclusion, 18% asymptomatic school going children in the study area were found to be carriers of *Campylobacter coli*, *Campylobacter jejuni* and *Campylobacter lari*, 84% of these

were multidrug resistant. More work on children carrying *Campylobacter* is needed to establish possibilities of previous exposure and virulence patterns of the *Campylobacter* isolates need to be investigated. Multidrug resistance in *Campylobacter* need to be addressed at all levels, the World Health Organization has recommended a multi-tiered and goal-oriented approach to control *Campylobacter* infections in both human and animals. Appropriate measures need to be taken to prevent *Campylobacter* transmission, including contaminated water and milk, through chlorination and pasteurization, respectively. Poultry, as the major reservoir, must be the main target in addressing human *Campylobacteriosis*⁴.

Data availability

Figshare: Multidrug resistant *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter lari* isolated from asymptomatic school going children in Kibera slum, Kenya.xlsx. <https://doi.org/10.6084/m9.figshare.11302292>³⁷.

File ‘Multidrug resistant *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter lari* isolated from asymptomatic school going children in Kibera slum, Kenya.xlsx’ contains the bacterial species identified from samples, the antibiotic zones of inhibition and the presence or absence of antibiotic-resistance genes in each sample.

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Acknowledgements

The authors are grateful to the Grand Challenge Canada and the Swedish research Council VR for funding this study; the pupils, teachers and parents of the study schools; The contribution of the technical members of staff Department of Public Health Pharmacology & Toxicology and the staff of Peepoople Kenya is highly acknowledged.

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Version 2

Reviewer Report 13 June 2023

<https://doi.org/10.5256/f1000research.29094.r176399>

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Maria Pavlova 

National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria

The conclusions are partly drawn and inadequately supported by the results. It is needed data about the contact people of Campylobacter-positive children. Are they examined for Campylobacter? Are only the children in their families positive for Campylobacter or any of their relatives?

The second essential point is the reservoir of Campylobacter sp. How many of the Campylobacter carriers share a home space with chickens? And if Yes, why these chickens weren't examined to confirm a relatedness between the Campylobacter strains? Have Campylobacter-positive confirmed children a diarrhoea history soon before the research?

I advise the authors to compare the laboratory-confirmed campylobacter incidences with the laboratory-confirmed carriers by season, sex and age distribution, administrative areas etc. To present more epidemiological data and analysis.

And second piece of advice are to present more suggestions for the reason for such a high tetracycline resistance- mass usage in feeding farm animals or mass usage in human medicine etc.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Enteric infectious diseases, diagnosis, outbreak surveillance & management, AMR.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 12 October 2020

<https://doi.org/10.5256/f1000research.29094.r70872>

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Musafiri Karama 

Veterinary Public Health Section, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa

The paper is scientifically sound in its current form and only minor, if any, improvements are suggested

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular epidemiology of Foodborne pathogens: Pathogenic E. coli, Campylobacter and Salmonella and bacteriophages.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 21 July 2020

<https://doi.org/10.5256/f1000research.23456.r65988>

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Byeonghwa Jeon 

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Abstract:

1. Background: Should read: the antibiotic resistance patterns of
2. Results: antibiotic concentrations, not the amount, need to be used. This applies to the entire manuscript.

Introduction:

1. "It was not until recently that *Campylobacter* spp. was shown to exhibit multidrug resistance (MDR). Before that, the bacteria were considered to be susceptible" it may be difficult to say like this as the MDR was reported more than two decades ago.
2. Quinolone resistance determination region (QRDR).
3. Campylobacter does not have parC. Mutations in parC cause FQ resistance in E. coli and Salmonella; but this is not applied to Campylobacter.

Method:

1. Antibiotic concentrations, not the amount, are to be made known in the supplement.

2. Campylobacter is microaerophilic. 'Anaerobic conditions' --> 'Microaerobic conditions'.

3. Did the authors include a quality control strain to the antibiotic susceptibility test?

Results:

1. Figure 1: There is no positive control for C. lari

Others:

1. Did the asymptomatic children carrying Campylobacter have any previous experience of diarrhea? It may be an important point to discuss whether Campylobacter-positive children are completely asymptomatic carriers or were recovering from Campylobacter exposure without manifesting clinical symptoms.

2. Overall, it reads well, but there are some minor English issues in some parts.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

No source data required

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Antibiotic resistance of foodborne pathogens

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 27 March 2020

<https://doi.org/10.5256/f1000research.23456.r59720>

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The manuscript entitled “Multidrug-resistant *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter lari* isolated from asymptomatic school-going children in Kibera slum, Kenya” investigated the occurrence and antimicrobial resistance of *Campylobacter* spp. among school-going children in a populous slum of Kenya. The study is well executed but will need wordsmithing to improve its readability.

A weakness of this manuscript is the lack of recent citations in the reference list. I suggest the authors update the reference list by including at least more than 60% of references that have been published in the last 10 years or less. The conclusion will also have to focus on the topic of the research (*Campylobacter* prevalence and antimicrobial resistance in young children).

I have inserted in the manuscript my corrections and suggestions to improve its clarity and style. This can be found [here](#).

Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular epidemiology of Foodborne pathogens: Pathogenic *E. coli*, *Campylobacter* and *Salmonella* and bacteriophages.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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