Investigating Carriage, Contamination, Antimicrobial Resistance and Assessment of Colonization Risk Factors of *Campylobacter spp*. in Broilers from Selected Farms in Thika, Kenya.

Mwajuma K. Abubakar[1*], Anne W. T. Muigai[2], Perpetual Ndung'u[3] and Samuel Kariuki[4]

[1]Department of Medical Microbiology, College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, Nairobi Kenya

[2]Department of Botany, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

[3]Department of Medical Laboratory Sciences, College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, Nairobi Kenya

[4]Center for Microbiology Research, Kenya Medical Research Institute, Nairobi, Kenya

CORRESPONDING AUTHOR

Mwajuma Kantono Abubakar

Department of Medical Microbiology,

School of Biomedical Sciences, College of Health Sciences

Jomo Kenyatta University of agriculture and Technology

Juja, Nairobi-Thika Highway

P.O. BOX 62000 – 00200 Nairobi, Kenya

Phone: +254 724 379 280/ +254 736 690 702

Email: mwajumaabu@gmail.com

*+254 379 260/ +254 690 702 mwajumaabu@gmail.com

ABSTRACT

Aims: To investigate carriage and contamination rates of broiler meat, the factors that are associated with *Campylobacter spp.* colonization and its phenotypic and genotypic antimicrobial resistance from Thika small-scale poultry farms.

Study Design: The study design was cross-sectional and laboratory based, it employed simple random sampling across 18 small-scale farms.

Site and duration of study: The study was conducted between August and December 2017 at Thika sub-county, a town located 42 Km North East of Nairobi.

Methodology: One hundred and eighty five cloaca swab samples from live broilers and 158 neck swab samples from broiler carcasses were collected. Isolates were obtained by plating method using mCCDA, conventional methods and duplex PCR were used for the isolation and identification of *Campylobacter* species.

Results: Carriage prevalence was at 15.67%, significantly (P = .000) lower than contamination prevalence detected at 30.37%. While the overall *Campylobacter spp.* prevalence was 22.45%. Risk of *Campylobacter* colonization in the flock doubled in feeding broilers with chicken waste and older poultry, at (OR: 2.57, 95% CI: 0.19 - 34.47) and (OR: 2.00, 95% CI: 0.312 - 12.84) respectively. The *Campylobacter spp.* were resistant (P < .05) against Ciprofloxacin, Streptomycin, and Trimethoprim between carriage and contamination. MDR was 79.22%; XDR was 12.98% while no PDR recorded.

Conclusion: Broilers in Thika region are potentially important source of human infection and possible continuity of infection from the threat posed by *Campylobacter* carrier broilers. Presence of *sulI* and *dhfr* genes with high resistance observed for quinolones, sulfonamides, ß-lactams and trimethoprim, thus posing a major public health problem for consumers of poultry products.

Keywords: Carriage, Contamination, *Campylobacter spp.*, Duplex PCR, Multi drug resistance, Resistance genes

1. INTRODUCTION

Poultry are major reservoirs of *Campylobacter spp.* and thus the main source of human campylobacteriosis [1]. *Campylobacter jejuni* and *Campylobacter coli* are the two major species known to dominate in human campylobacteriosis [2]. This disease is the most common cause of bacterial gastroenteritis, with symptoms ranging from abdominal pain, fever, mild watery diarrhea to bloody stools [3]. Reiter's syndrome and Guillian-Barre syndrome may occur as complications in severe cases [4]. The epidemiology of *Campylobacter spp.* in poultry production is still incompletely understood [5]. For more than a decade, there has been a major debate on whether vertical or horizontal transmissions are responsible for introduction of *Campylobacter* into flocks [5, 6]. *Campylobacter* invade chicken early in life through various risk factors as several studies have shown revealing potential *Campylobacter* introduction channels into broilers houses as well as factors contributing to the introduction have been studied [7]. Risk factors that have been associated with *Campylobacter* ability to colonize chicken include but are not limited to contaminated drinking water, administration of antibiotics, [8, 9]; poor hygiene [10]; and old age of the flock [11]. Despite good hygiene practices, broiler slaughter poses a risk of cross-contamination and bacteria spread from the gastrointestinal tract to the carcass and subsequently to humans [12, 13]. The ISO method 10272-2 for food legislation purposes is the official method for detection and enumeration of *Campylobacter spp*. while the molecular methods are not considered "confirmatory" tests [14].

In Africa, epidemiology of *Campylobacter* (especially for *C. jejuni*, *C. coli* and *C. lari*) infection have not been sufficiently addressed due to lack of national surveillance program and most of the *Campylobacter spp.* estimate reports are mainly from laboratory-based surveillance of pathogens responsible for diarrhea [15]. However, few prevalence studies conducted on *Campylobacter* enteritis in five African states showed a range of between 5 to 20% [15]. In Brazil, contamination of chicken carcasses with *Campylobacter spp.* from various slaughterhouses was 16.8% where *C. jejuni* isolation was higher (93.8%) than *C. coli* [16]. While a Sri Lanka study [17] samples purchased at retail shops detected much higher *Campylobacter spp.* prevalence (59%) with *C. coli* being the most frequently isolated species at 69.2% than *C. jejuni* at 30.7%.

Recent study in Nairobi reported the following *Campylobacter* prevalence; between 33-44% for broiler chicken, 60% for indigenous chicken farms and 64% for chicken meat retailers from Dagoretti and Kibera informal settlement areas [18].

Although *Campylobacter* infections are self-limiting, in severe cases of prolonged enteritis and septicemia, antimicrobial treatment is often needed [19]. Fluoroquinolones and macrolides are often the drugs of choice to treat human campylobacteriosis. However, over the years studies have reported increases in resistance to Fluoroquinolones and Macrolides of *Campylobacter spp.* despite they being drugs of choice for its treatment.[20]. A study from Northern Tunisia showed very high resistance rates detected against quinolones, tetracycline and macrolides ranging from 88.6% to 100% [21].

Albeit Thika is one of the largest broiler suppliers to the capital, Nairobi, there is scanty information regarding this pathogen. To the best of our knowledge, this is the first study to document carriage, contamination and resistance prevalence including resistance genes of *Campylobacter* in broilers from small-scale farmers in Thika. In addition, the study evaluated factors that are associated with *Campylobacter* colonization consequently might have contributed to carriage, contamination and antibiotic resistance in this region.

2. MATERIALS AND METHODS

2.1 Sample collection

Thika is an industrial town located at 42 Km North East of Nairobi where intense broiler farming is widely practiced. Nairobi city is a major market for the poultry products. The study design was cross-sectional and laboratory based, it employed simple random sampling method where 343 samples were collected across 18 farms in Landless location between August and December 2017. One hundred and eighty five cloaca samples from live poultry while 158 neck swabs from broiler carcasses were collected for determination of carriage status and contamination respectively. Swabs with modified charcoal-cefoperazone-deoxycholate agar (mCCDA) were used for sampling and further transported in a box with ice packs to the laboratory where analysis were done immediately.

2.2 Culture, Isolation and Identification of Campylobacter

Samples were directly plated onto mCCDA and incubated at 42^{O}C for 48 h in a microaerophilic environment (5% O_2, 10% CO_2 and 85% N_2) generated by candles. Suspect *Campylobacter* colonies by

colonial characteristics were further identified by conventional methods (Gram stain, Oxidase, Catalase and hippurate tests), then emulsified in Eppendorf tubes with sterile distilled water ready for DNA extraction.

2.3 Identification by PCR

Polymerase Chain Reaction (PCR) assay was performed to identify *Campylobacter* genus prior to the duplex PCR to identify *C. jejuni* and *C. coli.* The *cadF* gene was selected as Universal forward primer, FU, (Amplicon size; 101 - 120) and reverse primer, R1, (Amplicon size; 478 - 497) described previously [22]. R2 (Amplicon size; 542 – 561) and R3 (Amplicon size; 818 – 837) for identification of *C. coli* and *C. jejuni* respectively [23].

Table 1: Primer Sequences for identification of cadF (Campylobacter genus), aspK (C. coli) and hipO (C. jejuni) Genes Used in Duplex Polymerase Chain Reaction

Primer	Primer sequence ('5 – 3')[i]	Product size, bp	Identification	Reference
FU	TTGAAGGTAATTTAGATATG	400	*Campylobacter* spp.	Konkel et al.
R1	CTAATACCTAAAGTTGAAAC	400	*Campylobacter* spp.	Konkel et al.
R2	TTTATTAACTACTTCTTTTG	461	*C. coli*	Shams S et al.
R3	ATATTTTTCAAGTTCATTAG	737	*C. jejuni*	Shams S et al.

[i] 43^{O}C annealing temperature for all the primers

DNA extraction by boiling for 25min in a water bath at 100^{O}C followed by centrifugation for 15 min at 15000rpm was done and supernatant used for the analysis. Reaction tubes contained a final reaction volume of 25µl comprised of 4µl duplex PCR master mix, Betaine 1µl, 1µl primer (for each of the four primers) and 1µl DNA template. Amplification reactions were carried out in a thermocycler under the following conditions: initial denaturation for 3min at 95^{O}C 1 cycle; 32 cycles denaturation for 30s at 94^{O}C, annealing at 43^{O}C for 30s, extension for 30s at 72^{O}C and a final extension for 5min at 72^{O}C. The PCR products analyzed by electrophoresis on stained 1.5% agarose gel under UV light.

Levene's test of equal variance (t-test) was used to determine the statistical difference between carriage and contamination prevalence at $P = .05$.

2.4 Analysis of risk factors

Six variables were tested; hygiene practices (good, fair or poor), age of poultry (< 3weeks or > 3weeks), type of feed (kitchen waste, chicken feed or both), antibiotics used (tetracycline or none), rinse procedure (Bucket or running water) and slaughter area (open grounds, slaughter house or near poultry house), used to evaluate risk factors associated with *Campylobacter* colonization. Analyzed by odds ratio (OR) at 95% Confidence Interval (CI) and Chi square tests at $P = .05$.

2.5 Antimicrobial Susceptibility Test

Antimicrobial Susceptibility Tests (ASTs) of *Campylobacter* species were performed against 12 antimicrobial agents; Ampicillin 10µg (AMP), Gentamicin 10µg (CN), Tetracycline 30µg (TE), Erythromycin 15µg (E), Chloramphenicol 30µg (C), Trimethoprim 1.25µg (W), Sulphamethoxazole 23:75µg (RL), Nalidixic Acid 30µg (NA), Ofloxacin 5µg (OFX), Kanamycin 30µg (K), Streptomycin 10µg (S) and Ciprofloxacin 5µg (CIP) were used for this analysis based on the commonly used antibiotics in Kenya. Disk diffusion method [24] was carried out recommended by the Clinical Laboratory Standards Institute (CLSI, 2012) and European Union Committee for Antimicrobial Susceptibility Testing (EUCAST, 2017)). Mueller Hinton Agar number 2 (MHA-II) was used with sterile 5% defibrinated sheep blood to grow a lawn of the bacterial isolate from freshly prepared 0.5 McFarland inoculated on the MH-II and eventually impregnated with antimicrobial disks and incubated under microaerophilic conditions for 48h at 42^{O}C, according to a previous study [25].

Lists of antimicrobial breakpoints from the Centre for Disease Control & Prevention (CDC), European Centre for Disease Control (ECDC), the European Committee on Antimicrobial Susceptibility Testing (EUCAST), Clinical Laboratory Standards Institute (CLSI) and the United States Food and Drug Administration (FDA). Multi drug resistant (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, extensively drug resistant (XDR) was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories and pan drug resistant (PDR) was defined as non-susceptibility to all agents in all antimicrobial categories [26]. These were used

to categorize the isolates susceptibility and resistance as MDR, XDR or PDR from the measured zones of inhibition.

Statistical difference between carriage and contamination resistance was determined by Levene's test for equality of variance (t-test) $P = < .05$ followed by a non-parametric test (Mann Whitney U test) using a null hypothesis that stated; Distribution of antimicrobial agent is the same across the farms at significance level of 5% and 10%.

2.6 Determination of resistance genes

The highly resistant isolates against the various agents were selected for the characterization of their respective resistance genes (R-genes). Trimethoprim (*dhfr* gene), Sulfamethoxazole, (*sull* gene) and Nalidixic Acid (*gryA* gene) R-genes were characterized at 126bp, 223bp and 620bp respectively. There were no R-genes in Nalidixic Acid while characterization for Ampicillin was not done. Reaction tubes contained a final reaction volume of 25µl comprised of; 4µl PCR master mix 18µl PCR water, Betaine 1µl, 2µl primer and 1µl DNA template. Amplification reactions for *dhfr* and *gryA* genes in a thermocycler were under the following conditions; initial denaturation for 4min at 95^{O}C, 30 cycles denaturation for 1min at 94^{O}C, annealing at 60^{O}C for 1min, extension for 50s at 72^{O}C and a final extension for 5min at 72^{O}C. Same conditions applied for *sull* gene except for annealing which was at 65^{O}C.The PCR products were analyzed by electrophoresis in stained 1.5% agarose gel under UV light.

Nalidixic Acid resistance using *gyr*A F- 5' GCTCTTGTTTTAGCTTGATGCA-3'and R-'5 TTGTCGCCATCCTACAGCTA-3' with annealing temperature of 50˚C was used to detect PCR reaction product of 620bp.

Sulfamethoxazole R-genes were detected using primer set F- 5'CGCACCGGAAACATCGCTGCAC 3' and R- 5' TGAAGTTCCGCCGCAAGGCTCG 3' to amplify *sull* gene with annealing temperature of 65^{O}C to detect PCR reaction product of 223bp.

Trimethoprim R-genes were detected using primer set F-5' CATGGTTGGTTCGCTAAACTGC3' and R-5'GAGGTTGTGGTCATTCTCTGGAAATA 3' to amplify *dhfr* gene with annealing temperature of 60^{O}C to detect PCR reaction product of 126bp.

The PCR conditions were; denaturation at 95^{O}C for 4 min, 33 cycles with denaturation at 94^{O}C for 1 minute, annealing at varying temperatures; extension at 72^{O}C for 50 seconds, and a final extension at

72^{O}C for 5 min. The separation of PCR products were done by gel electrophoresis on Ethidium Bromide stained 1.5% agarose gel. (Vaishnavi *et al*., 2015).

C. jejuni ATCC 33560 and *C. coli* ATCC 33559 were used as positive controls while *E. coli* ATCC 25922 as negative control.

Table 2: Number and Percentage Resistance Spectra of the 77 Campylobacter spp. isolates against 12 antimicrobial agents tested

Antibiotic Name	No. of resistant *Campylobacter* in carriage	No. of resistant *Campylobacter* in contamination	No. of resistant *C. jejuni*	No. of resistant *C. coli*	No. of resistant mixed species	No. of resistant of other *Campylobacter spp.*	Overall Resistant *Campylobacter spp.* isolates
AMP	22/29 (75.9%)	30/48 (62.5%)	24/35 (68.6%)	18/25 (72%)	5/8 (62.5%)	6/9 (66.7%)	52/77 (67.5%)
CN	9/29 (31%)	4/48 (8.3%)	5/35 (14.3%)	6/25 (24%)	1/8 (12.5%)	1/9 (11.1%)	13/77 (1.7%)
S	17/29 (58.6%)	8/48 (16.7%)	13/35 (37.1%)	9/25 (36%)	1/8 (12.5%)	1/9 (11.1%)	25/77 (32.5%)
K	10/29 (34.5%)	10/48 (20.8%)	9/35 (25.7%)	3/25 (12%)	0/8 (0%)	2/9 (22.2%)	20/77 (25.9%)
TE	8/29 (27.6%)	4/48 (8.3%)	7/35 (20%)	4/25 (16%)	1/8 (12.5%)	1/9 (11.1%)	12/77 (15.6%)
C	4/29 (13.8%)	8/48 (16.7%)	6/35 (17.1%)	3/25 (12%)	0/8 (0%)	2/9 (22.2%)	12/77 (15.6%)
E	10/29 (34.5%)	13/48 (27.1%)	9/35 (25.7%)	8/25 (32%)	2/8 (25%)	3/9 (33.3%)	23/77 (29.9%)
NA	19/29 (65.5%)	28/48 (58.3%)	17/35 (48.6%)	20/25 (80%)	7/8 (87.5%)	7/9 (77.8%)	47/77 (61%)
CIP	13/29 (44.8%)	7/48 (14.6%)	12/35 (34.3%)	6/25 (24%)	1/8 (12.5%)	1/9 (11.1%)	20/77 (25.9%)
OFX	9/29 (31%)	10/48 (20.8%)	10/35 (28.6%)	7/25 (28%)	0/8 (0%)	2/9 (22.2%)	19/77 (24.7%)

RL	22/29 (75.9%)	47/48 (97.9%)	30/35 (85.7%)	15/25 (60%)	8/8 (100%)	9/9 (100%)	69/77 (89.6%)
W	27/29 (93.1%)	45/48 (93.8%)	32/35 (91.4%)	15/25 (60%)	8/8 (100%)	8/9 (88.9%)	72/77 (93.5%)

3. RESULTS

3.1 Carriage Prevalence

This study recorded overall *Campylobacter* prevalence of 22.45%, 30 of the *Campylobacter spp*. confirmed by PCR while the rest 47 were positive by conventional methods. Test for equality of variances (t-test) P = .05 was used to determine significant difference between isolates confirmed by PCR and isolates identified by conventional methods where: ($T_{6.150}$ = 1.902, *P* <.05) at *P* = .11).

Carriage recorded a prevalence of 15.67%, Six (20.68%) of these confirmed by PCR and the remaining 23 (12.43%) by conventional methods. Isolation prevalence of the different *Campylobacter spp*. was 44.8%, 41.4%, 6.9% and 6.9% for *C. jejuni*, *C. coli*, mixed species and other *Campylobacter spp*. respectively.

3.2 Contamination Prevalence

Contamination recorded a prevalence of 30.37% where the statistical difference between carriage and contamination prevalence was at *P* = .000. C*. jejuni* was the predominant *Campylobacter* spp. at 41.6% followed by *C. coli* at 33.3%, mixed species at 10.4% and other *Campylobacter spp.* at 14.6%. The statistical difference of *C. jejuni* and *C. coli* between carriage and contamination was at *P* = .000.

3.3 Associated Risk Factors

All factors showed increased risk of *Campylobacter* colonization in the flock apart from two; hygiene practices and feeding the broilers with combination of chicken feed and kitchen waste. The highest risk was feeding broilers with kitchen waste and age of poultry which doubled the risk of *Campylobacter* colonization in the flock (OR: 2.57, 95% CI: 0.19-34.47, *P* = .46) and (OR: 2.00, 95% CI: .312-12.84, *P* = .46) respectively. Followed by slaughtering in the open ground (OR: 1.86, 95% CI: 0.28-12.31, *P* = .51) then slaughtering around the poultry house (OR: 1.25, 95% CI: 0.20-7.61, *P* = .80).

3.4 Antimicrobial Susceptibility Tests

The isolates showed increased resistance against Ampicillin, Nalidixic Acid, Sulfamethoxazole and Trimethoprim at 67.5%, 61%, 89.6% and 93.5% respectively. Isolates under Tetracycline and Chloramphenicol showed low resistance both at 15.6% with isolates under Gentamycin presenting the lowest resistance at 1.7%.Sstatistical difference of resistance between carriage and contamination was at; $P = .01$ in Sulfamethoxazole, $P = .01$ in Streptomycin and $P = .000$ at Ciprofloxacin. Among the six variables using Tetracycline in their broiler flock as growth promoters and prevention of infections recorded OR: 0.875 95% CI: 0.96-7.952 $P = .96$.

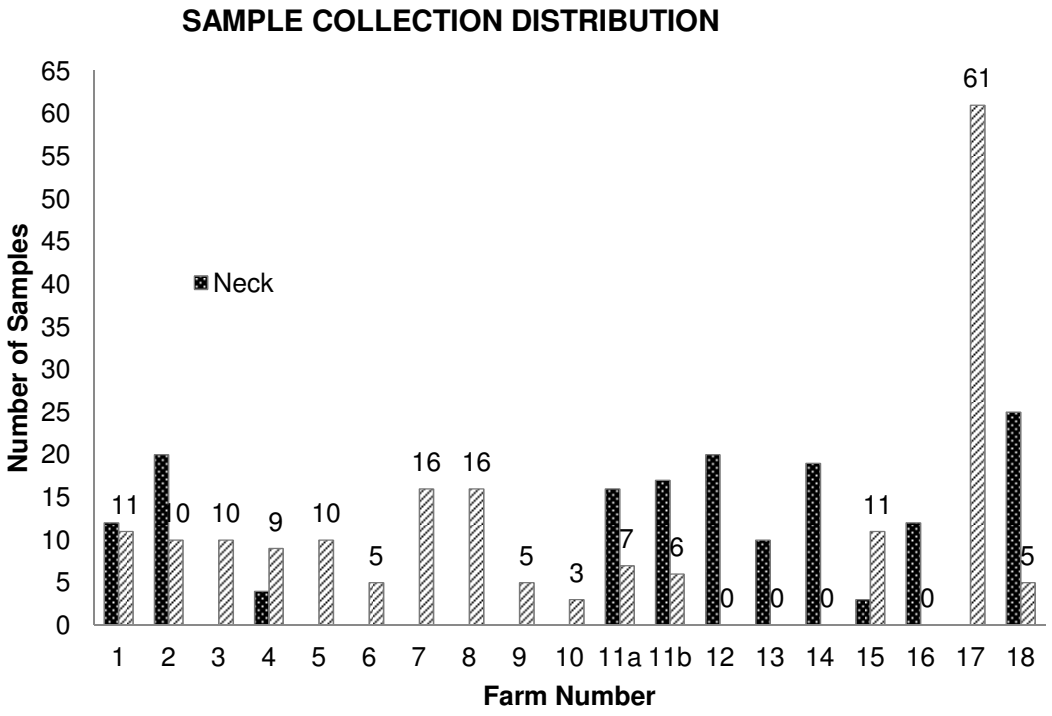

Figure 1: Graph pattern of sample collection distribution across 18 farms in Thika sub-County

The Mann Whitney U test was conducted in two categories, first category; *Campylobacter spp*. with very high resistance at $P = .05$ which included Ampicillin, Nalidixic acid, Sulfamethoxazole and Trimethoprim. From these, only Sulfamethoxazole ($P = .00$) null hypothesis was rejected. Second category; the other eight remaining antimicrobial agents tested with levels of significance of $P = .05$ followed by $P = .1$.

Streptomycin, Ciprofloxacin and Ofloxacin recorded the same *P* values from the two different levels of significance at

Table 3: Percentage Prevalence of Positive Campylobacter spp. isolated per farm across the 18 sampled farms in Thika

	Contamination		Carriage		
Farm No.	No. of Positive Samples	% Prevalence	No. of Positive Samples	% Prevalence	Total No. of Samples
1	10/12	**83.33%**	4/11	**36.36%**	23
2	1/20	**5%**	2/10	**20%**	30
3	No sample	**-**	0/10	**0%**	10
4	1/4	**25%**	3/9	**33.33%**	11
5	No sample	**-**	0/10	**0%**	10
6	No sample	**-**	0/5	**0%**	5
7	No sample	**-**	4/16	**25%**	16
8	No sample	**-**	6/16	**37.5%**	16
9	No sample	**-**	2/5	**40%**	5
10	No sample	**-**	3/3	**100%**	3
11 (a)	4/16	**25%**	1/7	**14.28%**	23
11 (b)	0/17	**0%**	0/6	**0%**	23
12	7/20	**35%**	No sample	**-**	20
13	0/10	**0%**	No sample	**-**	10

14	3/19	**15.79%**	No sample	**-**	19
15	0/3	**0%**	1/11	**9.09%**	14
16	4/12	**33.33%**	No sample	**-**	12
17	No sample	**-**	3/61	**4.92%**	61
18	18/25	**72%**	0/5	**0%**	30
TOTAL	**48/158**		**29/185**		**343**

P = .01, *P* = .00 and *P* = .05 respectively therefore their null hypothesis were rejected in both levels. Gentamycin (*P* = .07) null hypothesis was only rejected at *P* = .1 level of significance.

There was higher resistance prevalence of *C. jejuni* than *C. coli* (Table 2) in all the antimicrobial agents except Erythromycin, Nalidixic Acid and Ampicillin. The highest resistance of *C. jejuni* was 91.4% and 85.7% against Trimethoprim and Sulfamethoxazole respectively; Chloramphenicol had the lowest resistance prevalence (17.1%) in *C. jejuni*. While in *C. coli* Nalidixic Acid, was highest (80%) followed by Ampicillin (72%) and the lowest resistance was against Kanamycin and Chloramphenicol both at 12%.

The antibiotic susceptibility profile was studied to detect and profile MDR, XDR and PDR bacteria from Thika. MDR prevalence was 79.22% from this 36.06% represented MDR in carriage while MDR in contamination was much higher at 63.93%. In addition, MDR for *C. jejuni*, *C. coli*, mixed species of *C. jejuni*/*C. coli* and for other *Campylobacter spp*. was 44.26%, 32.78%, 13.11% and 9.83%% respectively. Isolates exhibiting XDR was 12.98%; with a 50/50 prevalence for both carriage and contamination isolates. The XDR distribution in the species was *C. jejuni* (50%); *C. coli* (40%), Other *Campylobacter spp*. (10%) and none for mixed species. Six isolates were found to be "just resistant" by the fact that the isolates were non-susceptible to only two antimicrobial agents. Thirty three percent represented resistant isolates in carriage while 66.66% represented the resistant isolates in contamination, with even distribution of 33.33% in *C. jejuni*, *C. coli* and other *Campylobacter spp*. while there was no isolates recorded for mixed species and no PDR isolates detected.

3.5 Resistance genes Characterization

dhfr gene was the most prevalent with seventeen R-genes compared to ten from the *sull* gene. There was 50% prevalence of the R-genes across the 18 sampled farms; Farm 18 had the highest prevalence,

40% of the resistance genes (only *dhfr* genes) while majority of the farms had just 3.70% prevalence. No R-genes were found in Nalidixic Acid-resistant isolates (*gryA* gene) while in Trimethoprim-resistant isolates characterization was not done. Farm 1 had two isolates while Farm 16 had one isolate carrying both *dhfr* and *sulI* genes. Distribution of *Campylobacter spp.* for *dhfr* gene was 17.64%, 23.52%, 29.41% and 29.41% for *C. jejuni*, *C. coli*, mixed species and other *Campylobacter spp*. respectively. While *sulI* gene recorded 30% for *C. jejuni*, 30% for *C. coli,* 30% for other *Campylobacter spp*. and only 10% for mixed species.

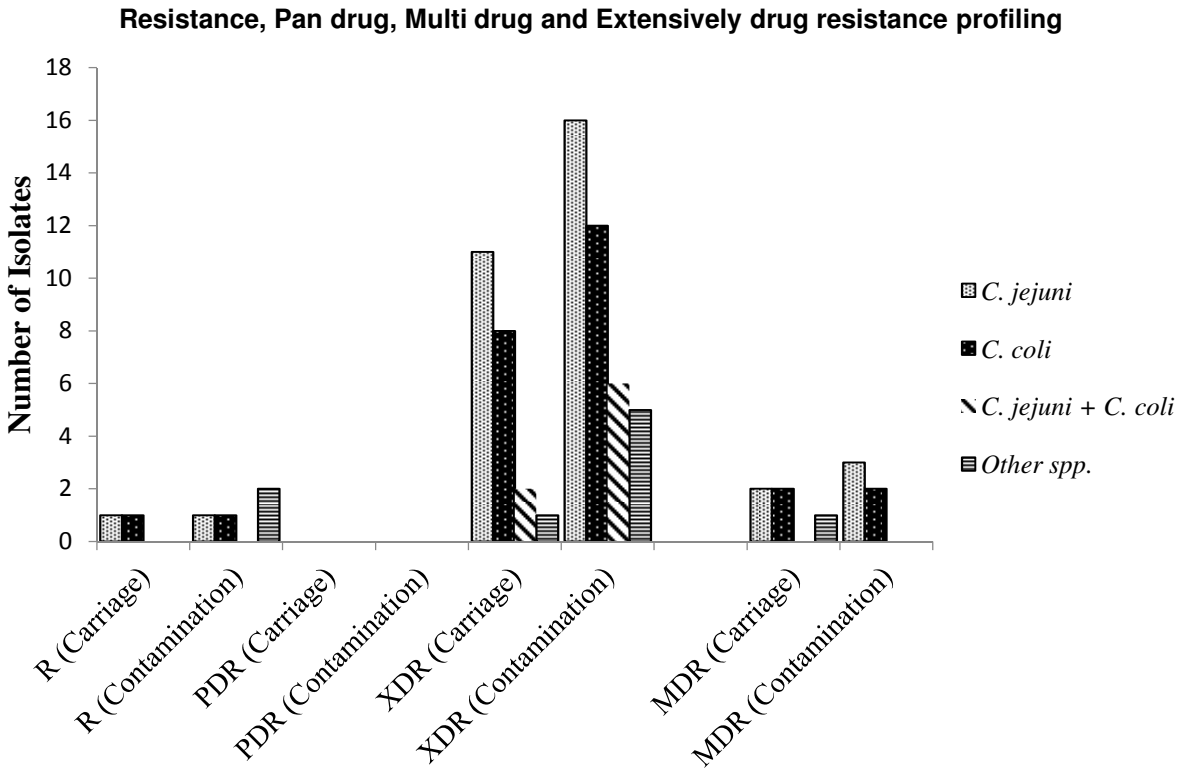

Figure 2: Antibiogram profile depicting antimicrobial susceptibility test (R, PDR, MDR and XDR) for *Campylobacter spp*. in carriage and contamination isolate

4. DISCUSSION

Thika sub-county is one of the largest broiler meat suppliers to the capital of the country, Nairobi where fried chicken is the fastest growing business thus, increasing the demand of broiler meat without

knowledge of the thermophilic bacteria that may come with it. This study recorded an overall *Campylobacter* prevalence of 22.45%. Unlike other studies in the sub-Saharan African countries, they recorded up to 47-68% [27, 28]. Which might be due to the small number of broiler farms sampled, a difference in size of commercial flocks, or a difference in sampling unit or even the testing methods. Recording carriage prevalence of 15.67% corroborating results from Ethiopia [29] that detected *Campylobacter* carriage with 18.41% prevalence in the Oromia region of the country and in 2013, 21.97% prevalence of *Campylobacter* from cloacal swabs was isolated in Italy [30]. In contrary, 42.5% prevalence of chickens (various breeds) by cloacal swabs was recorded from a study in Tanzania [31] and as high as 100% prevalence of *Campylobacter* in cloacal swabs was also found by direct counting on two types of agar in Brazil [32]. Further, *Campylobacter* spp. in carriage cases from the present study were identified; 44.8%, 41.4%, 6.9% and 6.9% for *C. jejuni*, *C. coli*, mixed species and other *Campylobacter spp*. respectively.

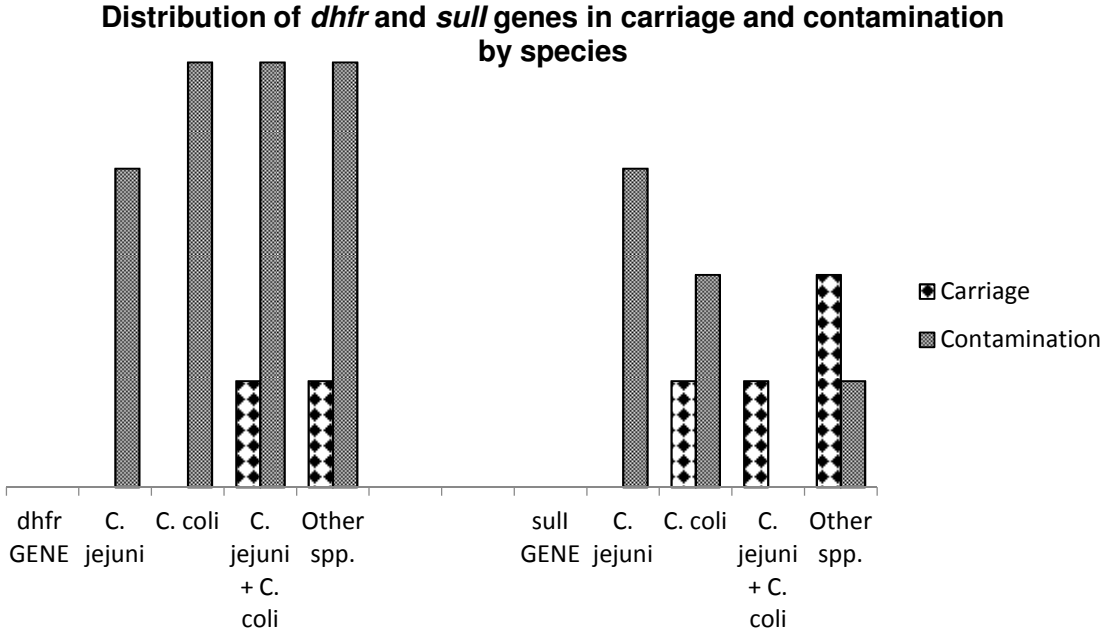

Figure 3: Chart depicting dhfr gene and sull gene distribution of Campylobacter species in carriage and contamination

These results conform to results reported by various studies; the prevalence of *C. jejuni* is usually higher than that of *C. coli*. Of the three species, *C. jejuni* predominates, with *C. coli* and *C. lari* infrequently recovered from the intestinal tract of poultry [33].

Farm 17 had the highest number of samples collected but with the least *Campylobacter* isolation prevalence at 4.9% in carriage cases. Contrary to Farm 10, which had, the lowest number of samples collected had 100% (3/3) *Campylobacter* isolation prevalence.

With 30.37% contamination prevalence (doubling carriage prevalence), this study recorded a higher contamination prevalence in comparison to few other studies that identified much lower prevalence; 21.7% in retail raw chicken meat tested in Ethiopia [34], and 21.9% of commercial chicken carcasses swabbed in Ghana [35].

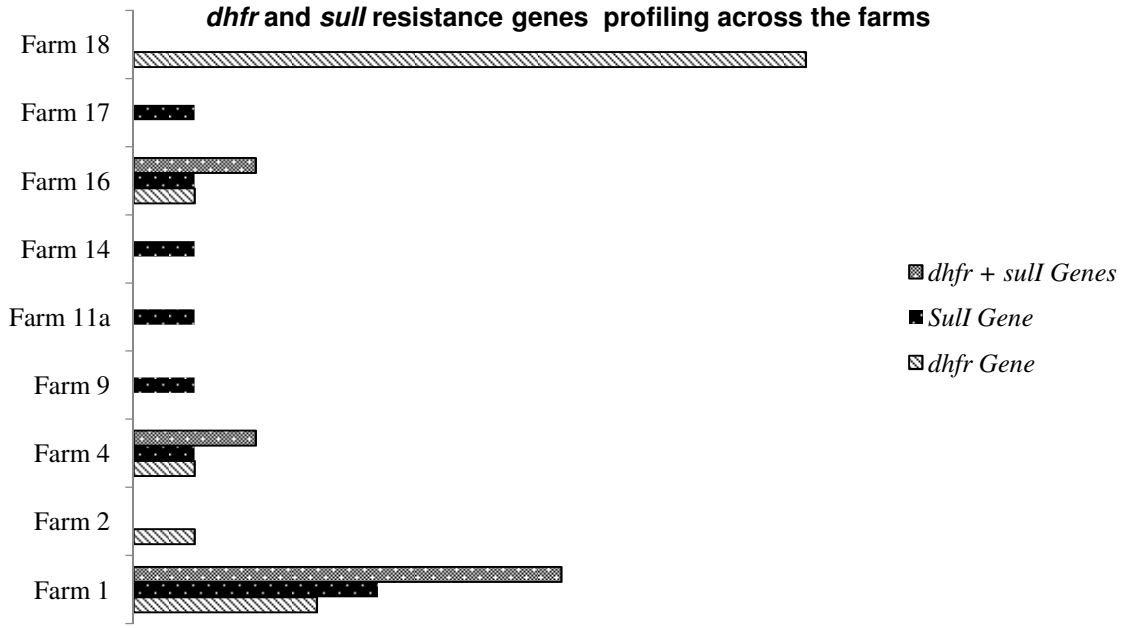

Figure 4: Resistance genes profiling of *dhfr* genes and sull genes across the 18 sampled farms.

However, much lower than the prevalence in a 2018 study a contamination prevalence of 91.07% in broilers was found in peri-urban areas of Nairobi [36] and 85.3% contamination prevalence was recorded in chicken meat from Nairobi tested less than 24hours after slaughter from supermarkets and butcheries [37]. *Campylobacter spp.* identification for contamination cases from this study revealed that *C. jejuni* was more predominant (41.6%) than *C. coli* (33.3%), these results corroborated with results from southern

Brazil where samples from the broiler slaughtering process recorded *C. jejuni* as the most predominant species at 72% and 38% for *C. coli*. Similarly, *C. jejuni* is responsible for over 95% of the diagnosed cases of campylobacteriosis as discussed earlier in Gonsalves' work in 2016. Notably, samples might contain multiple *Campylobacter* species, suggesting mixed colonization [38].

Farm 1 had highest number of contamination cases (83.3%) with 66.6% *C. coli* and 33.3% *C. jejuni*, with other *Campylobacter spp*. at only 10% species isolation prevalence.

Consistent with [39] prevalence of and risk factor for *Campylobacter* in France, the present study showed hygiene practices in Thika farms could contribute to a reduction in *Campylobacter* colonization, a factor found to have the lowest risk in this study. Feeding the broilers with kitchen waste and age of poultry doubled the risk of *campylobacter* colonization in the flock followed by slaughtering in the open ground then slaughtering around the poultry house. On the other hand, a combination of the chicken feed and kitchen waste showed a much-reduced risk compared to as when the broilers were fed on either of the two feeds. The farmers seemed to maintain good standards of hygiene practices apart from a few cases that did not raise the level of risk as usually expected.

Campylobacter infections cause gastroenteritis which is typically self-limiting the most important treatment is to avoid dehydration. Antibiotics treatment is usually needed in the most severe and persisting infections or pregnant women, young children, the old as well as immunocompromised patients [40, 41]. There is strong evidence to support the observation the fluoroquinolone use in food animals is associated with increased numbers of infections with resistant strains of *Campylobacter* in humans [42]. Interestingly, Australian livestock does not utilize fluoroquinolones and as a result, *Campylobacter* isolates from this region have negligible levels of resistance to fluoroquinolones, which in turn correspond to low resistance levels in human isolates [43]. November 30, 2018 reports; Canada took a major step to stop antibiotic resistance on farms by implementing new regulations for access to antibiotics for farm animals, starting December 1, 2018 farmers in Canada will have access to 300 animal drugs only if they obtain a prescription from a veterinarian (https://qz.com/1480983/antibiotic-resistance-on-farms-could-be-slowed-by-canadas-new-regulations/.

Generally, there was high resistance prevalence in this study and even higher resistance in isolates against Ampicillin, Nalidixic Acid, Sulfamethoxazole and Trimethoprim at 67.53%, 61.03%, 89.61% and

93.50% respectively (Table 2). These results are in accordance with resistance investigation of *Campylobacter* isolates from Kenyan chicken [44] where high resistance (>70%) was found in Nalidixic Acid, the same was observed in China [45].This wide-spread resistance to Nalidixic Acid corroborated reports on *Campylobacter* from di□erent food animals/products in other countries [46, 47]. In contrary, [48] reported lower Nalidixic Acid resistance rates (26%) for *Campylobacter* recovered from humans with diarrhea in Western Kenya in 2006. Similarly, high resistances of various proportions of Trimethoprim-Sulfamethoxazole [48, 49] have been reported in Kenya. These Ampicillin-resistant isolates results are also consistent with [50] in South Korea, recorded 88.9% Ampicillin resistance in all the *C. coli* isolated in ducks in 2014 and a similar trend in 2015 was recorded (75.7%) in Tanzania [51]. Gallay and colleagues [52] found the proportion of resistance to Ampicillin increased among the groups of patients in that study. Ampicillin is of clinical interest because at times is used for the treatment of severe campylobacteriosis. There was moderate resistance from the 77 *Campylobacter* isolates against Ciprofloxacin (25.97%), Kanamycin (25.97%), Ofloxacin (24.67%), Erythromycin (29.87%) and Streptomycin (32.46%) (Table 2). Unlike many studies with high fluoroquinolones resistance [50, 53, 54],Ciprofloxacin and Ofloxacin resistance was much lower in this study, while no resistance to fluoroquinolones was found in Tanzania [55]. Generally, Macrolides are now considered the optimal antibiotic for treatment of *Campylobacter* infections; however, resistance to macrolides in human isolates in some countries is becoming a major public health concern. The macrolide resistance among *Campylobacter* strains has remained low and stable level for a long while. However, there is also evidence in some parts of the world that resistance rate to Erythromycin, and other macrolides in these bacteria are slowly increasing [56].

Much lower resistance in this study were recorded against Tetracycline 15.6%, Chloramphenicol 15.6% and Gentamycin 1.7%. The Tetracycline results corroborate the results by Brooks and others from Western Kenya in 2006, where 18% prevalence was obtained, contrary to this, 10 years later Nguyen and colleagues recorded >70% resistance against Tetracycline.

The Mann-Whitney U test rejected the hypothesis that distribution of Sulfamethoxazole, Streptomycin, Ciprofloxacin and Ofloxacin are the same across the farms at $P = .05$ level of significance, also rejected the same hypothesis in Gentamycin, Streptomycin, Ciprofloxacin and Ofloxacin at $P = .01$ level of significance.

There was generally higher resistance prevalence in *C. jejuni* than in *C. coli* (Table 2) in all the antimicrobial agents except for Erythromycin, Nalidixic Acid and Ampicillin. The highest resistance in *C. jejuni* was 91.4% and 85.7% were recorded as the highest resistances against Trimethoprim and Sulfamethoxazole respectively; Chloramphenicol had the lowest resistance prevalence (17.1%) against *C. jejuni*. While Nalidixic Acid was highest (80%) followed by Ampicillin (72%) and the lowest resistance was in Kanamycin and chloramphenicol both at 12% against *C. coli* (Table 2). However, [57] reported low level of multidrug resistance in *C. jejuni* from broilers of the member states of the EU.

MDR prevalence in the present study was 79.22% from this 36.06% represented MDR in carriage while MDR in contamination was much higher at 63.93%. In addition, MDR for *C. jejuni*, *C. coli*, mixed species and for Other *Campylobacter spp*. was 44.26%, 32.78%, 13.11% and 9.83%% respectively. In contrast, (40% *C. jejuni* and 69.9% *C. coli*) are comparable to those reported in other countries [58-60]. Isolates exhibited 12.98% XDR; with a 50/50 prevalence for both carriage and contamination isolates, species distribution was 50% *C. jejuni*, 40% *C. coli*, Other *Campylobacter spp*. (10%) and none for mixed species. Six isolates were found to be "just resistant" by the fact that the isolates were non-susceptible to only two antimicrobial agents. Thirty three percent (33.33%) represented resistant isolates in carriage while 66.66% represented the isolates in contamination, there was even distribution of 33.33% amongst *C. jejuni*, *C. coli* and other *Campylobacter spp*. while there was no isolates recorded for mixed species of *C. jejuni* and *C. coli*. There were no PDR isolates profiled in this study. These results are consistent with MDR observed in the majority of the tested isolates (94%) in a study conducted by Wang and colleagues, [61]. However 4.5% isolates were pan susceptible to all antimicrobials tested in Tanzania, according to Kashoma and colleagues.

Trimethoprim, *dhfr* gene and Sulfamethoxazole, *sulI* gene were characterized at 126bp in 17 isolates and at 223bp in 10 isolates respectively. No R-genes were found in Nalidixic Acid (*gryA* gene at 620bp) while in Ampicillin the characterization was not done. R-genes conferring resistance in the other antimicrobial agents against *Campylobacter spp*. were not investigated due to lack of enough resources faced by the study.

5. CONCLUSION

The prevalence results suggested that Thika has low broiler *Campylobacter* infection and that carriage prevalence was lower than contamination prevalence. These findings suggest that should the farmers in Thika stop feeding their broilers with kitchen waste; and slaughtering the broilers at relatively younger age, the broilers would be at a lower risk of *Campylobacter* colonization. High level of resistance against Nalidixic acid, Ampicillin, Sulfamethoxazole and Trimethoprim as well as multidrug and extensively drug resistance were recorded in this study while no PDR isolates were recorded. The R-genes analysis was of significance since the results corroborated results from the phenotypic resistance analysis of the *Campylobacter* isolates observed in the antimicrobial susceptibility tests. The resistance results of especially ß-lactams and quinolones is indication for the need to strengthen implementation of control procedures and antibiotic regulations to reduce antibiotic resistance. Thika broilers are potentially important source of human infection, awareness best achieved by educating the public and training farmers on best practices.

ACKNOWLEDGEMENTS

We the authors wish to express our utmost gratitude to Kenya Medical Research Institute, Center for Microbiology Research (KEMRI-CMR) for enabling sample processing and analysis in their laboratories and the technical support of their members of staff. We are also grateful to Amina Galata, Cyrus Kathiiko, Eric Odoyo and Ernest Wandera of Nagasaki University Institute of Tropical Medicine-Kenya Medical Research Institute (NUITM-KEMRI) Nairobi station and Dr. R. Mwakubambanya of Egerton University for all their technical/resource assistance and advice.

Thika sub-county veterinary officer Dr. Njoki for assistance accorded during data acquisition and lastly, the local veterinary and Ministry of Livestock and Agriculture offices for permission to study the area.

The study was self-sponsored.

COMPETING INTERESTS

The authors have declared that no competing interests exists.

AUTHOR'S CONTRIBUTIONS

MKA and SK contributed equally in the development of the study idea, study design and acquisition of data. MKA, SK, AWTM and PN all contributed in data analysis and interpretation of results. Manuscript development, its revision, writing and approval of paper contributed by MKA, SK, AWTM and PN.

CONSENT

All authors declare that written informed consent was obtained from the participating farmers before sample collection and for publication of the research findings.

ETHICAL APPROVAL

All authors hereby declare that principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed. All experiments have been examined and approved by the Kenya Medical Research Institute – Scientific Ethical Review Unit (KEMRI-SERU) and Center for Microbiology - Scientific Steering Committee (CMR-SSC) under code: KEMRI/SERU/CMRP00056/3506.

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