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Research Article
Assessment of Antimicrobial Resistance Profiles of *Campylobacter* spp. in Commercial Broiler Production Systems in Kenya

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Abstract

Background and Objective: Campylobacter is a common contaminant in foods of animal origin. Poultry products, which are the most common cause of human bacterial gastroenteritis worldwide, are an important economic activity in Kenya. The purpose of this study was to assess the prevalence and antimicrobial resistance (AMR) profiles of Campylobacter in intensively managed commercial broiler production systems in Kenya for three commonly used antibiotics (ciprofloxacin, tetracycline and erythromycin) and to characterize the genetics of the various species. **Materials and Methods:** Cloacal swabs were collected randomly from 600-day-old chicks at the hatchery and from 300, 33-day-old market-ready broilers from farms in six counties in Kenya. Bacterial cultures were evaluated for morphological characteristics. **Results:** There was no microbial growth observed from the swabs of day-old chicks. The prevalence rates observed for market-ready broilers ranged from 82-98%. Six Campylobacter species were isolated and *Campylobacter jejuni* was the most prevalent (73.8%). The isolates showed AMR rates of 94.6-97.8%, with significant differences across the counties in zone diameters for ciprofloxacin and 100% resistance to erythromycin and tetracycline. The broilers were reared in six counties of Kenya, with flock sizes of 12,000-18,000 in intensively managed production systems with experienced managers and supervisors. **Conclusion:** Campylobacter species were absent in day-old broiler chicks but showed a high prevalence rate in market-ready broilers from commercial large-scale production systems. Five Campylobacter species were identified and *C. jenuni* was the most prevalent. The isolates also exhibited high resistance levels to the tested antimicrobials.

Key words: Campylobacter, *C. jejuni* prevalence, antimicrobial profiles, antimicrobial resistance, commercial broiler production, Kenya

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antimicrobial resistant-microbes are found in humans, animals, food and the environment. In many places, antibiotics are overused and misused in both humans and animals, which encourages the spread of antimicrobial-resistant organisms[1]. Antimicrobial resistance (AMR) threatens the effective prevention and treatment of an ever-increasing range of infections that are caused by microorganisms and it is, therefore, an increasingly serious threat to global public health.

Campylobacter infections are generally mild but can be fatal among children under 5 years of age, the elderly and immune-suppressed individuals[2]. Bacteria normally inhabit the intestinal tract of warm-blooded animals such as poultry and cattle and are frequently detected in foods derived from these animals. In developing countries, Campylobacter infections in children less than 5 years of age are especially frequent, sometimes resulting in death[3]. The Campylobacter genus comprises microaerophilic Gram-negative bacteria that are frequently found in raw meat, particularly chicken and they are a significant cause of food poisoning following handling of raw or undercooked meat. *Campylobacter jejuni* is now recognized as one of the main causes of bacterial food-borne disease in many developed countries[4]. Campylobacter species are found in abundance on poultry farms and their surrounding environment, including the soil, water sources, dust, building surfaces and the air[5]. Poultry are also an important reservoir of other Campylobacter species, such as *Campylobacter lari*, *Campylobacter upsaliensis* and *Campylobacter concises*[6]. Domesticated broiler and imported chickens both contribute to the overall burden of Campylobacter infections[7]. It has been estimated that 71% of human Campylobacteriosis cases in Switzerland between 2001-2012 were attributed to chicken[8]. Because *C. jejuni* strains survive in chicken feces for up to 6 days after excretion, chicken feces may also be a potential source of transmission to the environment or humans when poultry manure is used as a fertilizer[9]. The United Kingdom Food Standards Agency reported preliminary findings showing that 72.9% of fresh whole retail chicken surveyed during 2014-2015 were infected with Campylobacter, with 18.9% of these harboring a level of >10,000 CFU g^{-1}, which is considered to be highly contaminated[10]. In a study conducted in Denmark among a flock of 162 chickens, Campylobacter spp. were isolated from 100% of organic broiler flocks, 36.7% of conventional broiler flocks and 49.2% of extensive indoor broiler flocks. Six of 62 Campylobacter isolates were resistant to one or more of the antimicrobials that were tested[11].

Chicken in Kenya is a popular source of protein and broiler production is a major economic activity. Kenya has an estimated 37.3 million birds, comprising free-range indigenous birds (84.1%; 31.4 million), layers (8.4%; 3.1 million) and broilers (5.7%; 2.1 million), while other poultry accounted for 1.8% (0.7 million). Poultry contributes about 55% to the livestock sector and represents 30% of the agricultural Gross Domestic Product (GDP). Broilers in Kenya are kept mainly in urban areas and the commercial poultry sector is estimated to produce over one million chicks per week. The features of the commercial broiler market are a growing urban population, growing retail sector such as fast foods, supermarkets and restaurants. The demand for commercial chicken (whole, half, parts, grilled and fried chicken) and eggs is high and increasing. The main broiler abattoir in Kenya has a throughput of over 7 million broilers annually.

Importance of the topic: The rapid emergence of resistant bacteria occurring worldwide is endangering the efficacy of antibiotics, which have transformed medicine and saved millions of lives. Many decades after the first patients were treated with antibiotics, bacterial infections have again become a threat even in developed countries[12]. The antibiotic resistance crisis has been attributed to the overuse and misuse of these medications, as well as the lack of new drug development by the pharmaceutical industry because of reduced economic incentives and challenging regulatory requirements[13].

Campylobacter causes diarrhea (often bloody), fever and abdominal cramps. Serious complications such as temporary paralysis can also occur and physicians rely on ciprofloxacin and azithromycin to treat patients with severe disease. However, Campylobacter has been reported to show resistance to these antibiotics[14]. Improper use of antibiotics, such as sub-lethal doses in feed to promote growth in livestock especially in poultry and pigs, self-medication with failure to complete the full dose in humans and the unnecessary use of antibiotics for such conditions as colds or flu have greatly contributed to the increasing resistance to antibiotics that is observed among several bacteria that have been, until recently, very susceptible to the particular drugs that were specified for their treatment[15]. Multidrug resistant Campylobacter and several other bacteria are now a major global concern[14]. Poor control of the use of antibiotics in under-resourced countries coupled with weak AMR surveillance makes mapping of resistance to antibiotics difficult.

Gaps in previous research: Kenyan studies have reported the occurrence of Campylobacter in poultry and poultry products from indigenous chicken[16,17]. Commercial broiler production is an important economic enterprise in Kenya and antimicrobials are commonly used. However, the prevalence and AMR profiles of Campylobacter species have not been analyzed in large scale commercial broiler production systems in Kenya. Studies have shown that the combination of tylosin and phosphomycin, as well as tetracycline and quinolone are the antibiotics of choice during the broiler rearing periods in large-scale and small-scale commercial farms in peri-urban Nairobi[18]. Information on the Kenyan situation regarding intensively produced large-scale commercial broilers was scarce and the aim of this study was to shed more light on the situation. However, a study on free-range indigenous chicken from some locations of Makueni County of Kenya[16] and another on live chickens in peri urban Nairobi[17] found that the prevalence rates of the genus Campylobacter were 50.87 and 70.6%, respectively. Lower rates have been reported in Tanzania[19] and Iran[20] and on Malaysian broiler farms[21].

The European Union flock prevalence rate is also lower than observed in this study[22]. Other researchers have reported high resistance levels to both ciprofloxacin and tetracycline in Campylobacter isolates from small scale and backyard chickens in Kenya[23]. In addition, a study in Western Kenya reported resistance to ciprofloxacin and tetracycline for Campylobacter isolates from human diarrheal cases[24].

Rationale to conduct the study: A study on small scale and backyard chickens recorded high resistance rates for ciprofloxacin, nalidixic acid and tetracycline with more than 70% resistance levels recorded[25]. Other researchers have investigated Campylobacter that was recovered from humans with diarrhea in Western Kenya and they found resistance rates for ciprofloxacin, nalidixic acid and tetracycline of 6%, 26 and 18%, respectively[24]. However, there is little information on the Kenyan situation regarding intensively produced large-scale commercial broilers in terms of Campylobacter prevalence and AMR profiles.

Purpose of the study: This study was designed to fill the above-mentioned gap, to assess the prevalence and AMR profiles of Campylobacter in intensively managed commercial broiler production systems in Kenya to three commonly used antibiotics (ciprofloxacin, tetracycline and erythromycin) and to genetically characterize the various species to inform the broiler industry and create awareness about the development of resistant strains that may pose a threat to public health.

MATERIALS AND METHODS

Experimental sites: Six contractual commercial farms, one located in each of the six counties in Kenya (Kajiado, Machakos, Murang'a, Kiambu, Nairobi and Nakuru) were included in this study. The farms were coded by county, had flock sizes of 12,000 to 18,000 birds each and were within a 100-km radius of the broiler processing plant.

Materials and research tools: A semi-structured questionnaire was used to establish the awareness and general knowledge of antimicrobial drugs and their use in the selected commercial broiler farms. Cloacal bacterial swabs were used for the 600-day-old broiler chicks and the 300 33-day-old market-ready broilers. A sterile cloacal swab was swirled 2-3 times along the cloacal wall and excess fecal material shaken off and then dipped into transport media(Stuart® transport media, Oxoid, Hampshire UK). The splint was then cut off at the brim of the vial using scissors dipped in 70% ethanol, to allow tight closure of the vial. The media vial was then placed in a cooler box with ice packs for shipment to the laboratory. In the laboratory the swabs were streaked onto modified cefoperazone charcoal agar mCCDA, (Oxoid, Hampshire, UK), which comprised agar plates with supplement (polymyxin B 2500IU, rifampicin 5 mg, trimethoprim 5 mg and cycloheximide 50 mg) and then incubated at 42°C for 48 h under anaerobic conditions. The mCCDA culture media (Oxoid, Hampshire, England) had been prepared according to manufacturer's instructions and stored at 4°C until use. After 48 h of incubation, the plates were checked for characteristic growth. Characteristic colonies (grey/white or creamy grey in color 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.

Experimental design: The protocol involved a cross-sectional survey that involved random collection of cloacal swabs from day-old-chicks at the hatchery at different times over a period

of 3 months and random sampling from market ready broilers of age 33-36 days until the required sample sizes were acquired.

Research procedure

Knowledge about antimicrobial use: A semi-structured questionnaire was administered to establish the general knowledge about drug use, the types of drugs that were used on the selected broiler farms and compliance with withdrawal periods before the slaughter of the birds. The manager and supervisor or proprietors of the farm were interviewed.

Hatchery visits and sampling of day-old chicks: Hatchery visits were performed early in the morning on the night after the chicks hatched. Strict hygiene and bio-security measures were used, including taking a complete shower and dressing in sterile clothing that was provided by the hatchery management. On each sampling day, 50 birds were randomly selected and sampled until 600 samples were collected. A sterile swab was inserted and rotated in a circular motion 3-4 times along the inner circumference of the cloaca. The end of the swab was then dipped into Stuart® transport media (Oxford, UK) and the wooden splint cut off to allow total immersion of the whole swab and tight closure of the vial. A pair of scissors dipped in 70% ethanol was used to cut the wooden splint. The sample vial was then clearly labeled to indicate the date of collection, county code to where the birds would be dispatched and serialized sample number and the samples were stored in a cooler box. The samples were transported to the laboratory at the Faculty of Veterinary Medicine, Department of Public Health, Pharmacology and Toxicology (PHPT), University of Nairobi, where laboratory analysis performed.

Sampling of market-ready broilers: Market-ready broilers from participating farms were sampled at the poultry meat processing plant before slaughter. Fifty broilers from each of the farms were randomly selected and cloacal swabs that were collected from each bird were handled as previously described for day-old chicks.

Bacterial cultures and isolation: Bacterial cultures were made by streaking (single-line streak inoculation) cloacal swab specimen onto petri-dishes containing mCCDA. The plates were then placed into a large glass jar with a candle at the bottom. The candle was then lit and the jar covered until the candle flame was extinguished. This method creates a micro-erophilic environment that is suitable for growth of Campylobacter spp[26]. The jar was then placed in the incubator at 42°C for 48 h, which represents ideal conditions for thermophilic Campylobacter growth.

Antimicrobial sensitivity testing: The Kirby-Baeur disk diffusion protocol[27] was used where antimicrobial disks (Becton Dickinson and Company, Franklin Lakes, NJ, USA) containing ciprofloxacin, tetracycline and erythromycin were placed onto the Mueller–Hinton agar (AldrichchemieGmbH, Taufkirchen. Germany)with pure cultures of the isolates. The plates were then incubated for 24 h at 37°C. The resultant zones of inhibition diameters, as visualized by the unaided eye, were measured using calipers to the nearest millimeter and recorded. These diameters were then interpreted using the European Union Committee on Antimicrobial Sensitivity Testing (EUCAST) standard to classify the organisms as sensitive, or resistant[28].

The zone diameters were reliably measured and the maximum number of disks on a plate depended on the size of the plate, the organism and the antimicrobial agents that were being tested. The number of disks on a plate was limited so that unacceptable overlapping of zones was avoided. A maximum of six disks were accommodated on a 90 mm circular plate and 12 disks on a 150 mm circular plate. After incubation, inhibition zones were read at the point where no obvious growth was detected by the unaided eye when the plate was held about 30 cm from the eye. The inhibition zone diameters were measured to the nearest millimeter using a caliper. The Mueller-Hinton agar plates were then read from the front with the lid removed and with reflected light. Zone diameters were interpreted and categorized as susceptible, or resistant according to the EUCAST clinical breakpoint tables[28].

Molecular characterization of the bacterial isolates: For DNA preparation, three distinct colonies from pure bacteria cultures were lifted with a sterile wire loop and suspended in 0.5 mL sterile, distilled water. The suspension was boiled for 30 min in a water bath. After cooling to room temperature, the preparation was centrifuged at 2000×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 six specific species were also identified: *C. coli*, *C. jejuni*, *C. lari*, *C. upsaliensis*, *C. hyointestinalis* and *C. fetus*. 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 min, 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[29]. were used to amplify a 812 bp fragment within the 16S rRNA gene of Campylobacter species using forward primer C412F 5′-GGATGACACTT TTCGGAGC-3′ and reverse primer; C1228R 5′-R-CATTGTAGC ACGTGTGTC-3′.Multiplex PCR was carried out with specific forward and reverse primers as follows *: C. jejuni* -forward-C1 5′- CAAATAAAGTTAGAGGTAGAATGT -3′; reverse-C3 5′- CCATAAGCACTAGCTAGCTGAT-3 (161 bp[30]), *C. coli* forward-HYO1F5′-ATAATTCTAGGTGAGAATCCTAG-3′, reverse-HYOFET23SR5′-CTTCGCATAGCTAACAT-3′(502 bp[30]), *C. fetus* forward-MG3F 5-GGTAGCCGCAGCTGCTAAGAT-3′,reverse-CF359R 5′- GCCAGTAACGCATATTATAGTAG-3′, (359 bp,[31,32]), *C. lari* forward-CLF 5′TAGAGAGATAGCAAAAGAGA-3′, reverse-CLR 5′-TACACATAATAATCCCACCC 3′(251 bp,[30]), HYO1F 5′-ATAATCTAGGTGAGAATCCTAG-3′, *C. hyointestinalis* forward-HYOFET23SR reverse-5′GCTTCGCATAGCTAACAT-3′ (611 bp[32]) and *C. upsaliensis* forward-CU61F 5′-CGATGATGTG CAAATTGAAGC-3′, reverse-CU146R 5'-TTCTAGCCCCTTGCT TGATG-3′ (86 bp[33]). 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. The PCR products were visualized using a digital camera (Genetics analysis grade, Fisher Scientific, NJ., USA) after 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.

Data Collected: The farm workers' demographic data and their knowledge level and use of antimicrobial agents on the broiler commercial farms was established. The prevalence, types and molecular characterization of Campylobacter species in the farms were identified. AMR profiles of Campylobacter resistance to commonly used antimicrobials in the production system were identified.

Statistical analysis: Data from the questionnaires were checked for accuracy and corroboration. The level of education of the persons involved in day-to-day management of the farm was extracted during the interview and tabulated. Data from laboratory work was first tabulated in MS Excel (Microsoft Corporation, Redmond, Washington DC, USA) showing date of collection, sample identity and county code. The data were then imported into SPSS statistical package (International Business Machines Corporation, Armonk, NY., USA) for analysis. The prevalence rates of the genus Campylobacter from the six counties were calculated using MS Excel and a horizontal bar graph was used to illustrate the results. Data on the six species of Campylobacter that were identified using the multiplex polymerase chain reaction (MPCR) method was also tabulated in MS Excel and the prevalence per county was illustrated using bar graphs. AMR profiles were recorded in an MS Excel work sheet. MPCR is a procedure used to amplify several different DNA sequences simultaneously using DNA in samples utilizing multiple primers and a temperature-mediated DNA polymerase in a thermal cycler. The primer design for all primer pairs were optimized to enable them to work at the same annealing temperature during PCR. The zones of inhibition diameters in millimeters were also tabulated in MS Excel and these were compared with the EUCAST clinical antimicrobial sensitivity standards. This standard interprets for the antibiotics ciprofloxacin, tetracycline and erythromycin. Interpretation for tetracycline is also used for doxycycline. The zonal diameters were statistically analyzed for the means and distribution proportions using Tukeys Honestly Significant Difference as well as F-statistic ($p<0.001$).

RESULTS

Demographic data: The farms were located within a 100 km radius of the processing plant in the different counties. The mean number of years of broiler rearing experience among the managers of the farms surveyed during the study was 12.5 years (range, 5.24-19.76 years), with flock sizes ranging from 12000 to 18000 ($\mu = 16788$) birds in batches of various ages. The mean training level among supervisors was college of animal health (level 3), while the managers, who at times were the proprietors, had tertiary level of education (level 4). The average number of workers who were directly involved in rearing of the flocks was four males and one female, with a range of 4-6 for males and 0-2 for females.

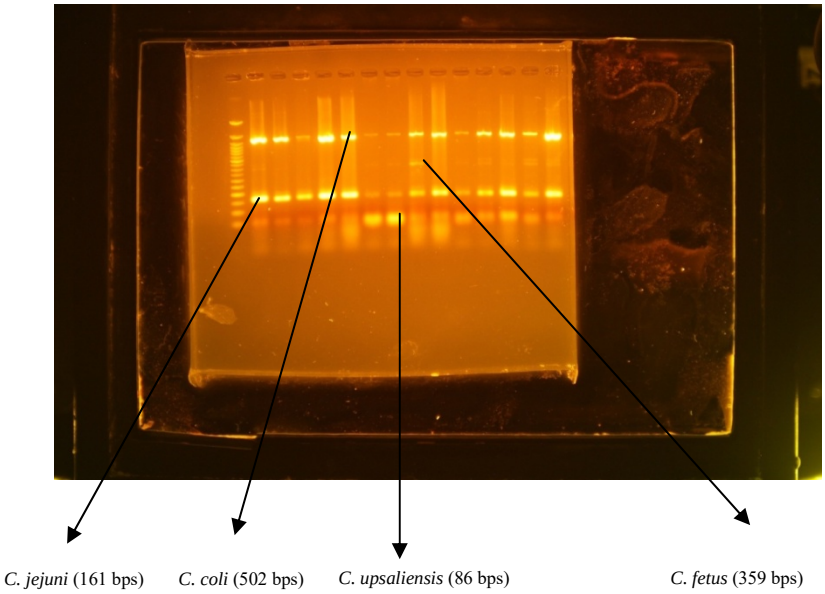

Fig. 1: Visualization of campylobacter species using multiplex PCR electrophoresis

Table 1: Prevalence and distribution of Campylobacter species isolated in the counties

	Sampled	Positive N (%)							
Countries	Total (N) km	Total isolates	*C. jejuni*	*C. fetus*	*C. coli*	*C. hyointestinalis*	*C. upsaliensis*	*C. lari*	Total (%)
Murang'a	50	50 (100.0)	40 (80.7)	2 (3.5)	5 (10.5)	0 (0.0)	2.65 (5.3)	0 (0.0)	100.0
Kiambu	50	49 (98.0)	37 (75.9)	10 (20.4)	2 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)	100.0
Nairobi	50	47 (94.0)	38 (75.6)	3 (4.9)	0 (0.0)	7 (14.6)	3 (4.9)	0 (0.0)	100.0
Machakos	51	46 (90.2)	36 (71.0)	12 (24.2)	0 (0.0)	1 (1.6)	0 (0.0)	2 (3.9)	100.0
Nakuru	50	44 (88.0)	33 (60.0)	0 (0.0)	15 (30.0)	5 (10.0)	0 (0.0)	0 (0.0)	100.0
Kajiado	50	41 (82.0)	44 (88.9)	0 (0.0)	2 (3.7)	4 (7.4)	0 (0.0)	0 (0.0)	100.0
Prevalence N (%)	301	277 (92.03)	222 (73.8)	30 (10.0)	27 (9.0)	15 (5.0)	5 (1.7)	2 (0.7)	100.0

Prevalence of campylobacter: All the samples (100%) from day-old chicks showed no growth on CCDA while 280 (80%) out of 300 samples from 33-day-old market-ready broilers showed growth of grey-white colonies that are characteristic of *Campylobacter* spp. On culture, Campylobacter was present in all the counties, with the highest prevalence of 100% in Murang'a County and the lowest prevalence of 82% from Kajiado County. Oxidase and catalase positivity was observed in all counties, at 91.6 and 91.3%, respectively.

Prevalence of campylobacter species isolated: Six species of Campylobacter were isolated as visualized by multiplex PCR electrophoresis (Fig. 1). *C. jejuni* was most prevalent and occurred in all counties, *C. fetus* and *C. coli* occurred at a moderate prevalence rate in four and three counties, respectively, while *C. lari* had the least prevalence and was only recorded in Machakos County. In summary, the average prevalence rates for the five species that were found in all counties were as follows: *C. jejuni*, 73.8%; *C. fetus*, 10%; *C. coli*, 9.0%; *C. hyointestinalis*, 5% and *C. upsaliensis*, 1.7% (Table 1).

A representative Multiplex PCR electrophoresis digital photo as it was seen at the PHPT laboratory, Kabete is presented in Fig. 1 and the bright bands corresponded to the base pairs of the respective Campylobacter species. The extreme left column is the molecular marker at intervals of 50 bps.

Antimicrobial sensitivity and resistance: The EUCAST clinical breakpoints indicate that diameters of 26, 24 and 30 mm are the minimum for classification of Campylobacter as sensitive to ciprofloxacin, 5 mg, tetracycline, 30 g and erythromycin, 15 mg, respectively. For bacterial culture with ciprofloxacin 5 g discs, the inhibition zone diameter ranged from 6-29 mm and there was a significant difference in zone diameters

Table 2: Inhibition zone diameters of Campylobacter isolates from 33-day-old market-ready broilers from various counties to ciprofloxacin, tetracycline and erythromycin

Antimicrobial			Ciprofloxacin			Tetracycline			Erythromycin		
Countries	N	Minimum	Maximum	%≥26 mm (sensitive)	%<26 mm (resistant)	Maximum	%≥30 mm (sensitive)	%<24 mm (resistant)	Maximum	% ≥24 mm (sensitive)	%<30 mm (resistant)
Kajiado	45	6	29	2.22	97.68	26	0	100	9	0	100
Kiambu	42	6	29	2.38	97.62	27	0	100	10	0	100
Machakos	46	6	18	0.00	100.00	25	0	100	10	0	100
Murang'a	46	6	24	0.00	100.00	26	0	100	12	0	100
Nairobi	37	6	29	5.41	94.59	27	0	100	8	0	100
Nakuru	45	6	22	0.00	100.00	23	0	100	10	0	100

across the counties. Kajiado, Kiambu and Nairobi showed that only 2.2, 2.4 and 5.4% of samples, respectively, had an inhibition zone diameter that was greater than 26 mm and thus interpreted as sensitive to ciprofloxacin, while samples from the rest of the counties exhibited 100% resistance to the same antimicrobial by showing zone diameters of 18, 24 and 22 mm. (Table 2). For tetracycline all the isolates were resistant with the maximum inhibition zone diameter ranging from 23-27 mm and none of the isolates were above the minimum cut off point of 30 mm in all the counties (Table 2). Results for erythromycin indicated that all the isolates were resistant having had inhibition zone diameters ranging from 6-12 mm and thus none was above the 24 mm mark for sensitive microbes. There was no significant difference in the resistance shown to erythromycin across the six counties (Table 2).

The means and the distribution of the inhibition zone diameters for the three antimicrobials tested are illustrated in Table 3. AMR of Campylobacter to ciprofloxacin, shown by zone diameters, had a μ of 9.92 mm (for N = 261, SE = 0.327; Table 3). However, the diameters were significantly different (F-value = 5.007; df = 5, 255; p<0.001) across the counties. Machakos, which had the lowest mean and Nakuru County appeared in both groups a and b. For tetracycline, the μ was 8.33 mm (N = 261, SE = 0.317) with no significant difference (F = 1.485, df = 5, 255 p<0.195), while for erythromycin, the μ was 6.37 (N = 261, SE = 0.065; F = 2.525; df = 5, 255; p<0.030) (Table 3).

The EUCAST clinical breakpoints indicate that diameters of 26, 24 and 30 mm are the minimum for classification of Campylobacter as sensitive to ciprofloxacin, tetracycline and erythromycin, respectively, meaning that the study showed high levels of antimicrobial resistance (AMR) to these antibiotics. In addition, the diameter for tetracycline also interprets for doxycycline, suggesting that the organism could also be resistant to it.

Table 3: The means and the distribution of the inhibition zone diameters to the three antimicrobials tested

	Mean±SE		
Antimicrobial	Ciprofloxacin	Tetracycline	Erythromycin
Countries			
Kajiado	10.78±0.86[b]	9.40±0.95	6.18±0.09[ab]
Kiambu	10.48±0.83[b]	8.17±0.73	6.38±0.16[ab]
Machakos	6.78±0.36[a]	7.41±0.64	6.37±0.14[ab]
Murang'a	11.70±0.79[b]	8.28±0.72	6.80±0.24[b]
Nairobi	10.16±1.01[b]	9.59±1.05	6.08±0.06[a]
Nakuru	9.76±0.71[ab]	7.36±0.53	6.36±0.15[ab]
F Statistic	5.007	1.485	2.525
p-value	0.001	0.195	0.03
Remarks	Highly significant	Not significant	Significant

Following separation of the means. [a]Group whose means showed no significant difference. [b]Group whose means showed significant differences

DISCUSSION

According to this study, the prevalence rate of campylobacter among market-ready broiler chickens from large scale commercial farms was over 92% on average and six species of the genus were genetically characterized using multiplex PCR method. There was no growth of any microbes observed from samples collected from day-old chicks, which ruled out horizontal transmission. This prevalence is much higher than that found in a study on free-range indigenous chickens from some locations in Makueni County, Kenya, where the prevalence was 50.87%[16]. The prevalence was also higher than that found in another study on live chickens in peri-urban Nairobi, which was 70.6%, suggesting that there was an increase in Campylobacter prevalence rates in the chicken populations in Kenya[17]. In Tanzania, researchers reported a lower prevalence rate of 69.8%[19]. On the Asian continent, researchers in Iran observed a prevalence rate of 76%[20], while another study on Malaysian broiler farms reported a slightly lower rate of 70%[21]. The European Union flock prevalence rate of 71.2%[22] was also lower than that observed in the current study.

Studies on prevalence and AMR of Campylobacter in intensively produced and highly managed broilers in Kenya are lacking. However, some studies among humans with cases of diarrhea have been documented. Campylobacter infection is a major cause of fatalities in children under 5 years of age and among adults, it is a precursor to Guillain-Barre syndrome, reactive arthritis (ReA) and traveler's diarrhea. Furthermore, according to this study, AMR to commonly used antibiotics such as ciprofloxacin, tetracycline, doxycycline and erythromycin was high (98-100%). The findings of the current study agree with a study on AMR of Campylobacter isolates from small scale and backyard chickens in Western Kenya[25]. In that study, 71% of isolates showed resistance to both ciprofloxacin and tetracycline. In the same region of Western Kenya, researchers have reported resistance levels to ciprofloxacin and tetracycline of 6 and 21%, respectively, in Campylobacter from human diarrheal cases. Resistance levels to erythromycin that were reported in the current study are in agreement with the findings of a study that was performed in Vietnam[25]. In a study from Northern Germany, Campylobacter isolates from fattened pigs demonstrated resistance to ciprofloxacin (range, 9.1-18.9%) and to tetracycline (range, 27.3-62.2%)[29]. In Spain, Campylobacter isolates found in chicken meat had resistance levels of 58.2% for ciprofloxacin and 1.8% for erythromycin[30]. Similar studies in Turkey found that the resistance to nalidixic acid and tetracycline in Campylobacter isolates from chickens was 95 and 56%, respectively[31]. These studies indicate that Campylobacter AMR profiles are prevalent both in humans and animals, thus, supporting the increasing concern about development of AMR among bacteria that are important to public health. Several studies have suggested that the indiscriminate and unregulated use of antimicrobials in agriculture and livestock production has contributed significantly to the development of AMR among bacteria. The use of antimicrobials on the farms that were involved in this study was strictly controlled and there was no routine use of drugs at sub-lethal doses for either prophylaxis or growth promotion. According to this study, all the broiler farms that were sampled reported having used Phosfomycin-tylosin (Fosbac®), oxytetracycline and quinolone at one time whenever the need arose and on the advice of technical teams. These antibiotics were only used if an infection occurred. The farms were found to be well serviced by technical teams from a common source as part of the contractual agreements and they had strict adherence to production protocols, with accurate and detailed record-keeping. All farms used the same phenol-based compound for disinfection of the poultry houses but Fosbac® was the antibiotic of choice when infections occurred. Loss of profits through carcass condemnations at the abattoir was a major driving force in strict observation of drug withdrawal periods among all the farms. This is in contrast to another study that was performed among small scale broiler farmers in peri-urban Nairobi, which reported many farmers failed to observe withdrawal periods, lacked free technical back-up and had limited knowledge of veterinary drugs. This led to residues in meat and likely contributed to AMR[18]. The hands-on managers were literate and well versed in the issues surrounding the use of veterinary drugs and their withdrawal periods.

Of great concern is the 100% resistance rate that was shown to both tetracycline and erythromycin and a significant resistance rate (98.47%) that was shown to ciprofloxacin by the Campylobacter isolates. According to a WHO report[32], selection for resistance in one part of the world affects health in other parts of the world through international travel and trade and *in vitro* antimicrobial susceptibility testing is essential to provide guidance to physicians and veterinarians on the appropriate treatment of infections and to generate data on the occurrence of acquired resistance in Campylobacter. Contamination of chicken meat may occur during processing or post slaughter handling but this aspect was not tested in the current study.

CONCLUSION

The study concluded that the prevalence of Campylobacter bacteria in market-ready commercial broilers was very high. Six Campylobacter species were identified and the isolates were also highly resistant to commonly used antibiotics such as ciprofloxacin, tetracycline and erythromycin, with no correlation between the drugs that were tested and those that were used on the farms that were studied. The day-old chicks had no antimicrobial profiles, which assured the production of quality chicks from the hatchery.

SIGNIFICANCE STATEMENT

This study showed the possible risk of high levels of Campylobacter contamination in commercial broiler chickens, which can be detrimental to human health; this is also a great public health concern. This study will help the researcher to uncover the critical area of AMR profiles in commercial broiler

production systems that many researchers were not able to explore. Thus, a new theory on the use of antimicrobials in commercial chicken production systems and possible linkage to cases of human campylobacteriosis, may be discovered.

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