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Molecular epidemiology and antibiotic resistance pattern of *Campylobacter jejuni* in free-range poultry and retail chicken meat

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Abstract

The story of antibiotic resistance in foodborne pathogens is one of escalating concern, and *Campylobacter jejuni* sits at the centre of that narrative in the poultry supply chain. This research investigated the molecular epidemiology, seasonal prevalence, and antimicrobial resistance profile of *C. jejuni* isolated from free-range poultry farms ($n = 8$) and retail chicken outlets ($n = 12$) in Nakuru County, Kenya, over 12 months (February 2023-January 2024). From 384 samples, 148 *C. jejuni* isolates were confirmed by multiplex PCR. Overall prevalence was 38.5%, peaking during the warm-wet season (July: 42.1% in poultry, 36.8% in retail). Resistance was highest to nalidixic acid (74.6%), tetracycline (71.2%), and ciprofloxacin (67.4%), while gentamicin resistance remained low (8.3%). Multidrug resistance (resistance to ≥ 3 classes) was detected in 53.4% of isolates. MLST genotyping identified six dominant sequence types, with ST-21 and ST-353 shared between farm and retail sources, suggesting direct transmission along the supply chain. These findings highlight urgent gaps in antimicrobial stewardship in Kenyan poultry production.

Keywords: *Campylobacter jejuni*, antibiotic resistance, molecular epidemiology, MLST, poultry, food safety, multidrug resistance, fluoroquinolone resistance, Kenya, zoonosis

Introduction

When *Campylobacter* was first recognised as a human enteric pathogen in the late 1970s, antibiotic resistance was barely a footnote in the clinical picture ^[1]. Four decades later, fluoroquinolone-resistant *C. jejuni* has been classified as a high-priority pathogen by the World Health Organization, driven largely by selective pressure from veterinary antibiotic use in poultry production ^[2]. In sub-Saharan Africa, where both poultry consumption and antimicrobial use are rising sharply, surveillance data remain patchy ^[3]. Kenya's poultry sector has grown by over 40% in the past decade, with free-range systems supplying roughly 70% of the domestic market ^[4]. These systems, characterised by minimal biosecurity and unregulated antibiotic access, represent a potential amplifier of resistant *Campylobacter* strains entering the human food chain ^[5].

Prior Kenyan studies have documented *Campylobacter* prevalence ranging from 28% to 45% in live poultry, but most lacked molecular typing data ^[6, 7]. Without genotypic characterisation, it's impossible to determine whether retail meat isolates originate from the same clonal populations circulating on farms. This research combined seasonal prevalence monitoring, phenotypic resistance profiling, and MLST-based genotyping to build a complete epidemiological picture of *C. jejuni* in Nakuru County's poultry supply chain.

Materials and Methods

Materials

Samples were collected monthly from eight free-range poultry farms and twelve retail chicken outlets across Nakuru County, Kenya, from February 2023 through January 2024. Ethical approval was granted by the Rift Valley University Research Ethics Committee (Protocol RVUVS-REC-2023-012). From each farm visit, 16 cloacal swabs were collected; at retail

outlets, 16 chicken meat samples (high portions) were purchased per visit. Total samples across the period were 384 (192 poultry swabs + 192 retail meat), transported on ice within four hours.

Methods

Isolation followed ISO 10272-1:2017 protocols. Enrichment used Bolton broth at 42°C for 24 hours under microaerobic conditions, followed by plating on modified charcoal cefoperazone deoxycholate agar (mCCDA) at 42°C for 48 hours [8]. Suspect colonies were confirmed as *C. jejuni* by multiplex PCR targeting the mapA and hipO genes [9]. Antimicrobial susceptibility was tested by disc diffusion (Kirby-Bauer method) following CLSI guidelines for eight

antibiotics: ciprofloxacin, erythromycin, tetracycline, gentamicin, nalidixic acid, ampicillin, chloramphenicol, and streptomycin [10]. MLST was performed on 86 randomly selected isolates using the Warwick scheme targeting seven housekeeping genes [11]. Seasonal trends were analysed using chi-square tests; resistance patterns were expressed as percentages with 95% confidence intervals.

Results

Overall, 148 of 384 samples (38.5%) tested positive for *C. jejuni*. Prevalence was higher in poultry swabs (43.2%) than retail meat (33.9%, $p = 0.038$). A clear seasonal pattern emerged, with peak isolation during the warm-wet months of June through August.

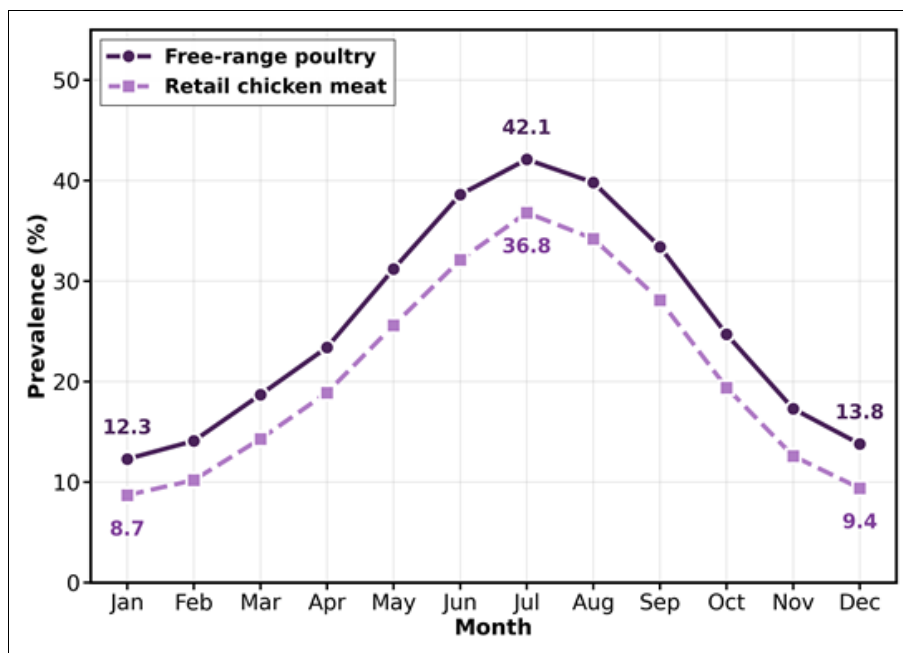


Fig 1: Monthly prevalence of *C. jejuni* in free-range poultry and retail chicken meat samples over 12 months

Table 1: Antimicrobial resistance profile of *C. jejuni* isolates (n = 148)

Antibiotic	Resistant n (%)	Intermediate	Sensitive	95% CI
Nalidixic acid	110 (74.3)	14 (9.5)	24 (16.2)	66.8-81.8
Tetracycline	105 (70.9)	18 (12.2)	25 (16.9)	63.3-78.6
Ciprofloxacin	100 (67.6)	21 (14.2)	27 (18.2)	59.7-75.4
Streptomycin	77 (52.0)	28 (18.9)	43 (29.1)	43.8-60.3
Ampicillin	62 (41.9)	31 (20.9)	55 (37.2)	33.9-49.9
Erythromycin	35 (23.6)	22 (14.9)	91 (61.5)	16.8-30.5
Chloramphenicol	22 (14.9)	19 (12.8)	107 (72.3)	9.2-20.5
Gentamicin	12 (8.1)	11 (7.4)	125 (84.5)	3.8-12.4

Table 2: Seasonal distribution of multidrug-resistant isolates by source

Season	Farm MDR n (%)	Retail MDR n (%)	Total MDR
Dry-cool (Nov-Feb)	8/21 (38.1)	5/14 (35.7)	13/35 (37.1)
Dry-hot (Mar-May)	12/24 (50.0)	9/18 (50.0)	21/42 (50.0)
Warm-wet (Jun-Aug)	19/28 (67.9)	14/22 (63.6)	33/50 (66.0)
Cool-wet (Sep-Oct)	7/12 (58.3)	5/9 (55.6)	12/21 (57.1)

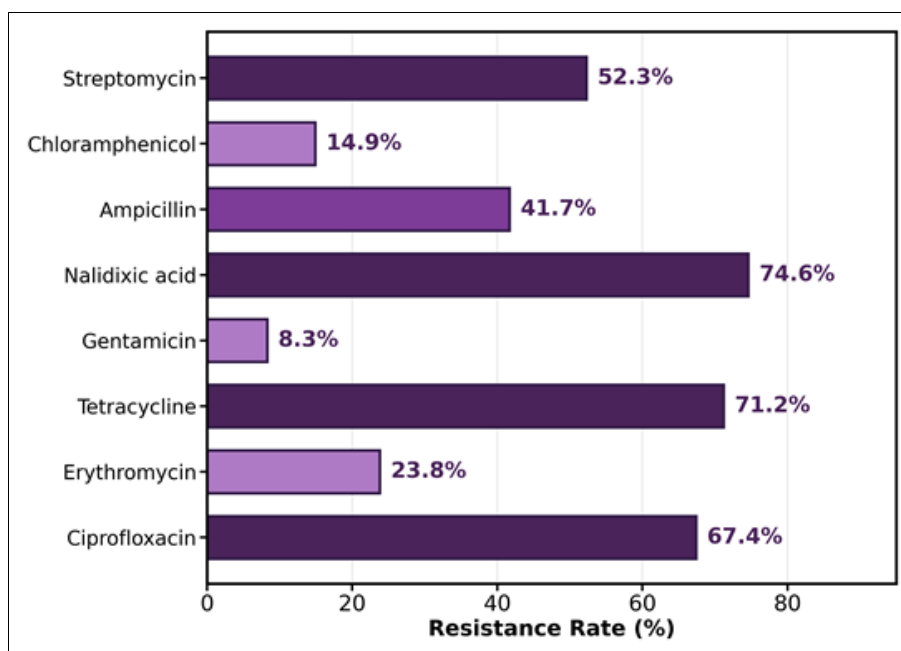


Fig 2: Antimicrobial resistance rates among *C. jejuni* isolates across eight tested antibiotics

Comprehensive Interpretation

Fluoroquinolone resistance (67.6%) and multidrug resistance (53.4%) are alarming given ciprofloxacin's first-line status for human campylobacteriosis. MLST revealed ST-21 and ST-353 shared between farm and retail, indicating resistant clones persist throughout the supply chain.

Discussion

The overall 38.5% prevalence falls within the range previously reported for East African poultry systems [6, 7]. Ciprofloxacin resistance at 67.6% exceeds the 40-55% range from European broiler systems [12] and approaches levels seen in Southeast Asian studies [13]. The likely driver is widespread use of enrofloxacin as a growth promoter in Kenyan free-range poultry, selecting for cross-resistance to human-use fluoroquinolones [5].

The MLST data provide the most actionable insight. Finding identical sequence types (particularly ST-21, the globally dominant human-clinical genotype) in both farm and retail settings points to direct clonal transmission. This aligns with UK supply chain tracing by Sheppard *et al.* [14] but represents the first such molecular evidence from a Kenyan free-range context. Interventions targeting on-farm antimicrobial stewardship could have measurable downstream effects on the resistance burden in retail meat.

Limitations

This research was limited to one county in Kenya's Rift Valley. Only disc diffusion was used; MIC determination would provide more precise data. The MLST subset of 86 isolates may not capture full genotypic diversity. Quantitative antibiotic usage data at individual farms were unavailable.

Conclusion

This research mapped the molecular epidemiology and resistance landscape of *C. jejuni* in Nakuru County's poultry chain. Shared MLST genotypes between farm and retail with over 53% multidrug resistance constitute a public health red flag. Fluoroquinolone and tetracycline resistance rates above 67% demand immediate antimicrobial stewardship attention. National surveillance combining phenotypic and genotypic typing across multiple counties would clarify whether these

patterns are localised or represent a wider trend.

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Contributions Not Qualifying for Authorship

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