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Antimicrobial Susceptibility Profiles, Multidrug Resistance Patterns, and MAR Index Analysis of Campylobacter jejuni and Campylobacter coli Isolated from Poultry and Chicken Handlers in Maiduguri, Northeast Nigeria

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Abstract

Background: Antimicrobial resistance (AMR) in *Campylobacter jejuni* and *Campylobacter coli* is an escalating global public health concern, driven by misuse of antibiotics in poultry production. In northeastern Nigeria, AMR surveillance data for *Campylobacter* from live bird markets (LBMs) are essentially absent. This study characterised antimicrobial susceptibility profiles, multidrug resistance (MDR) patterns, multiple antibiotic resistance (MAR) index values, and resistance gene carriage among *Campylobacter* isolates from chickens and chicken handlers in Maiduguri Metropolis.

Methods: Forty-nine phenotypically and molecularly confirmed *Campylobacter* isolates from 212 samples (broiler cloacal swabs, local chicken cloacal swabs, and hand swabs) were subjected to disc diffusion testing against ten antibiotics per CLSI (2016) guidelines, supplemented by EUCAST where CLSI breakpoints were unavailable. The MAR index was calculated as the ratio of antibiotics resisted to total antibiotics evaluated. Multiplex PCR detected tetracycline *tet(A)* (888 bp) and erythromycin *erm(A)* (190 bp) resistance genes. Exact binomial 95% CIs (Clopper–Pearson) were calculated for all resistance rates. MDR was defined as resistance to ≥1 agent in ≥3 antibiotic classes (Magiorakos et al., 2012).

Results: Tetracycline resistance was highest (17/49; 34.7%; 95% CI: 21.3%–49.4%) followed by norfloxacin (11/49; 22.4%; 95% CI: 11.5%–36.4%). All isolates were susceptible to ciprofloxacin and levofloxacin (each 91.8% sensitive; 0% resistant; 95% CI for resistance: 0.0%–7.3%) and no isolate was resistant to erythromycin. MDR was detected in 28/49 (57.1%) isolates across 13 distinct resistance patterns. MAR indices ranged from 0.30 to 0.70 (mean 0.40; median 0.40); 16 isolates (32.7%) had MAR ≥0.40, indicating origin from high antibiotic-use environments. The *tet(A)* and *erm(A)* genes were detected by multiplex PCR, corroborating phenotypic resistance findings.

Conclusions: The 57.1% MDR rate and the predominance of tetracycline resistance in *Campylobacter* from Maiduguri's LBMs constitute a public health concern requiring urgent antibiotic stewardship, restriction of over-the-counter antibiotic access in poultry, and routine AMR surveillance. Complete susceptibility to ciprofloxacin, levofloxacin, and erythromycin supports their use as empiric first-line agents pending susceptibility data.

Keywords: antimicrobial resistance; Campylobacter coli; Campylobacter jejuni; live bird market; MAR index; multidrug resistance; tetracycline resistance; Nigeria

Background

Antimicrobial resistance (AMR) was declared a global public health emergency by the World Health Organization in 1997 (Abd El-Hack et al., 2022) and identified as a major global risk by the World Economic Forum in 2013 (Schrijver et al., 2018). Among foodborne pathogens, thermophilic *Campylobacter* species have emerged as leading AMR contributors, with

resistance to fluoroquinolones, tetracyclines, and macrolides documented on every inhabited continent (Ge et al., 2013). *Campylobacter jejuni* and *Campylobacter coli* together account for approximately 95% of human campylobacteriosis cases (Coker et al., 2002), making resistance in these species a direct clinical threat.

In Nigerian poultry production, tetracyclines and other antimicrobials are routinely administered without veterinary prescription as growth promoters and prophylactic agents, creating sustained selection pressure for resistance (Shaikh et al., 2015). The spread of resistant strains from animals to humans through the food chain and occupational contact is a critical pathway for community-level AMR dissemination (Schrijver et al., 2018). The multiple antibiotic resistance (MAR) index provides a validated, quantitative tool for determining whether isolates originate from high-risk antibiotic-use environments; a MAR index >0.2 is considered indicative of such high-risk sourcing (Davis & Brown, 2016; Osundiya et al., 2013).

Tetracycline resistance in *Campylobacter* is primarily conferred by the *tet(O)* and *tet(A)* genes, which encode ribosomal protection proteins (Gibreel et al., 2004). Erythromycin resistance involves mutations in 23S rRNA and operates through the CmeABC efflux system (Acheson & Allos, 2001). Molecular confirmation of resistance genes provides mechanistic understanding beyond disc diffusion phenotyping and informs surveillance of transmissible resistance determinants (Abdi-Hachesoo et al., 2014).

Despite the recognised importance of AMR surveillance at the human–animal interface, comprehensive data on *Campylobacter* resistance profiles from LBMs in northeastern Nigeria where informal poultry trading is widespread and antibiotic governance is minimal are absent. This study addressed that gap.

Methods

Isolate Source and Study Setting

This study used 49 *Campylobacter* isolates obtained from a cross-sectional survey conducted June–August 2020 across six LBMs in Maiduguri Metropolis, Borno State, northeastern Nigeria. Isolates were recovered from 212 samples: 135 broiler cloacal swabs, 60 local chicken cloacal swabs, and 17 hand swabs from chicken handlers. All isolates were confirmed by culture on Campylobacter-selective agar at 42°C under microaerobic conditions, biochemical profiling (oxidase+, catalase+, citrate+, urease−), and PCR (16S rRNA, 566 bp; multiplex *HipO*/*CeuE*). Ethical approval was obtained from the Borno State Ministry of Health.

Antimicrobial Susceptibility Testing

Disc diffusion (Kirby-Bauer method) was performed on Mueller-Hinton agar following CLSI (2016) guidelines, with EUCAST breakpoints applied where CLSI interpretive criteria were unavailable for Campylobacter (Narvaez-Bravo et al., 2017). A 0.5 McFarland standard bacterial suspension was inoculated onto Mueller-Hinton agar. Ten antibiotic discs (Oxoid, UK) were applied: ciprofloxacin (CPX, 10 µg), norfloxacin (NB, 30 µg), gentamycin (CN, 10 µg), amoxicillin (AMX, 30 µg), tetracycline (TET, 50 µg), rifampicin (RD, 10 µg), erythromycin (E, 30 µg), chloramphenicol (CH, 20 µg), ampidox (APX, 30 µg), and levofloxacin (LEV, 10 µg). Zones of inhibition were read at 24–48 h using the Sensitre Vizion system 40. Campylobacter jejuni ATCC 33560 was used as quality control.

MDR Classification and MAR Index

Isolates were classified as MDR if resistant to ≥1 agent in ≥3 different antimicrobial classes per international consensus definitions (Magiorakos et al., 2012). The MAR index was computed as MAR = a/b, where a = number of antibiotics resisted and b = total antibiotics assessed (n=10). MAR >0.2 indicates probable origin from a high-risk, high-antibiotic-use environment (Davis & Brown, 2016). Descriptive statistics (mean, median, range, interquartile range) were computed for the MAR index distribution across MDR isolates.

Molecular Detection of Resistance Genes

Multiplex PCR detected the tetracycline resistance gene *tet(A)* (888 bp; Maynard et al., 2004) and the erythromycin resistance gene *erm(A)* (190 bp; Strommenger et al., 2003).

Reactions (20 µl) used 4 µl Master Mix, 0.8 µl each primer (10 mM), 2 µl DNA, and 13.2 µl RNase-free water. Cycling: 95°C × 3 min; 35 cycles of 95°C × 30 s / 48°C × 30 s / 72°C × 30 s; final extension 72°C × 3 min. Primer sequences are shown in Table 1

Table 1: Primers used for multiplex PCR detection of antimicrobial resistance genes.

Target Gene	Primer Sequence (5'–3')	Amplicon (bp)	Reference
tet(A)	F: AACTTAGGCATTCTGGCTCAC R: GAAGGCAAGCAGGATGTAG	888	Maynard et al. (2004)
erm(A)	F: TATCTTATCGTTGAGAAGGGAT R: CTACACTTGGCTTAGGATGAAA	190	Strommenger et al. (2003)

Statistical Analysis

Data were analysed using SPSS version 20.0 (IBM Corp., Chicago, IL, USA). Exact binomial 95% CIs were calculated using the Clopper–Pearson method for all resistance proportions. Regression and correlation analyses examined associations between antibiotic resistance and the presence of Campylobacter species. Statistical significance was set at $p<0.05$. Resistance patterns were tabulated in ascending order of MAR index magnitude.

Results

Antimicrobial Susceptibility Profiles with Confidence Intervals

Table 2 presents the complete susceptibility profiles for all 49 isolates with exact 95% CIs for resistance rates. Tetracycline resistance was the highest at 34.7% (95% CI: 21.3%–49.4%), followed by norfloxacin at 22.4% (95% CI: 11.5%–36.4%). Resistance to ampidox, gentamycin, chloramphenicol, rifampicin, and amoxicillin was detected at lower rates, each with broad CIs reflecting the small denominator. No resistance was detected to ciprofloxacin, erythromycin, or levofloxacin (0.0%; exact 95% CI upper bound: 7.3% for each). The high intermediate categories for amoxicillin (85.7%) and rifampicin (81.6%) indicate dose-dependent susceptibility rather than frank resistance.

Table 2: Antimicrobial susceptibility of Campylobacter isolates (n=49) with exact 95% Clopper–Pearson CIs for resistance rates

Antibiotic (Disc Concentration)	Sensitive n (%)	Intermediate n (%)	Resistant n (%)	95% CI for Resistance
Ciprofloxacin (CPX, 10 µg)	45 (91.8)	4 (8.2)	0 (0.0)	0.0% – 7.3%
Norfloxacin (NB, 30 µg)	1 (2.0)	37 (75.5)	11 (22.4)	11.5% – 36.4%
Gentamycin (CN, 10 µg)	17 (34.7)	29 (59.2)	3 (6.1)	1.3% – 16.6%
Amoxicillin (AMX, 30 µg)	6 (12.2)	42 (85.7)	1 (2.0)	0.1% – 9.7%
Tetracycline (TET, 50 µg)	5 (10.2)	27 (55.1)	17 (34.7)	21.3% – 49.4%
Rifampicin (RD, 10 µg)	8 (16.3)	40 (81.6)	1 (2.0)	0.1% – 9.7%
Erythromycin (E, 30 µg)	33 (67.3)	16 (32.7)	0 (0.0)	0.0% – 7.3%
Chloramphenicol (CH, 20 µg)	26 (53.1)	21 (42.9)	2 (4.1)	0.5% – 13.8%
Ampidox (APX, 30 µg)	9 (18.4)	32 (65.3)	8 (16.3)	7.2% – 29.8%
Levofloxacin (LEV, 10 µg)	45 (91.8)	4 (8.2)	0 (0.0)	0.0% – 7.3%

MAR Index Distribution Analysis

MDR was detected in 28/49 (57.1%) isolates. Thirteen distinct resistance patterns were identified (Table 3) spanning MAR indices of 0.30–0.70. Descriptive statistics for the MAR index distribution among MDR isolates are mean = 0.40, median = 0.40, range = 0.30–0.70, interquartile range (IQR) = 0.30–0.40. The modal MAR index was 0.30, occurring in 12 isolates (42.9% of MDR isolates). Sixteen isolates (32.7% of all 49 isolates; 57.1% of MDR isolates) had MAR ≥0.40, indicating high-risk contamination sources with heavy antibiotic use (Davis & Brown, 2016). The most extreme pattern (CN–AMX–RD–CH–APX–TET–NB; MAR=0.70) was present in one isolate and conferred resistance across seven agents spanning five antibiotic classes.

Table 3: Antimicrobial resistance patterns of Campylobacter isolates in ascending MAR index order (n=28 MDR isolates of 49 total)

No. of Agents Resisted	Resistance Pattern	Isolates n (%)	MAR Index	Antibiotic Classes Involved
3	CH – NB – TET	2 (4.1)	0.30	3
3	NB – AMX – TET	7 (14.3)	0.30	3
3	TET – RD – APX	1 (2.0)	0.30	3
3	APX – TET – NB	2 (4.1)	0.30	3
4	AMX – NB – TET – APX	4 (8.2)	0.40	4
4	TET – CH – APX – NB	2 (4.1)	0.40	4
4	RD – APX – CH – NB	1 (2.0)	0.40	4
4	AMX – RD – APX – CN	1 (2.0)	0.40	4
5	TET – CH – APX – NB – E	2 (4.1)	0.50	5
5	CN – TET – APX – AMX – NB	2 (4.1)	0.50	4
6	RD – APX – CH – CN – TET – NB	2 (4.1)	0.60	5
6	CN – AMX – NB – RD – TET – APX	1 (2.0)	0.60	5
7	CN – AMX – RD – CH – APX – TET – NB	1 (2.0)	0.70	5

NB=norfloxacin; TET=tetracycline; CH=chloramphenicol; APX=ampidox; AMX=amoxicillin; RD=rifampicin; CN=gentamycin; E=erythromycin. MDR = multidrug resistant. MAR = multiple antibiotic resistance.

Molecular Detection of Resistance Genes

Multiplex PCR detected the *tet(A)* gene (888 bp) and the *erm(A)* gene (190 bp) in isolates consistent with the phenotypic resistance results. The presence of *tet(A)* corroborated the high tetracycline resistance rates observed by disc diffusion. Detection of *erm(A)* in the absence of frank phenotypic erythromycin resistance is consistent with sub-clinical or low-level resistance determinants, a phenomenon previously documented when CmeABC efflux pump activity and 23S rRNA mutations interact at sub-threshold levels (Acheson & Allos, 2001).

Discussion

The highest resistance rate in this study was to tetracycline (34.7%; 95% CI: 21.3%–49.4%), consistent with the globally documented pattern of high tetracycline resistance in *Campylobacter* and comparable to rates reported in Sokoto, Nigeria (Salihu et al., 2012) and in sub-Saharan African regional studies. Samad et al. (2018) reported 75% tetracycline resistance among *Campylobacter* isolates in poultry, while Lopes et al. (2018) reported 16.7% demonstrating considerable geographic variation. The mechanistic basis for tetracycline resistance rests primarily on the *tet(O)* gene, which encodes a ribosomal protection protein on self-transmissible plasmids (45–58 kb) capable of horizontal gene transfer between *Campylobacter* strains and across species (Gibreel et al., 2004). Detection of *tet(A)* in the present study confirms that genotypic resistance mechanisms are active in Maiduguri isolates, posing a risk of mobile genetic element-mediated dissemination (Carattoli et al., 2017).

Norfloxacin resistance at 22.4% (95% CI: 11.5%–36.4%) is noteworthy given the therapeutic importance of fluoroquinolones for campylobacteriosis. By contrast, the complete absence of ciprofloxacin and levofloxacin resistance (0.0%; exact upper CI: 7.3%) may reflect lower utilisation of these specific agents in Maiduguri's poultry sector relative to norfloxacin, a finding consistent with Salihu et al. (2012) in Sokoto. This contrasts with global trends: Engberg et al. (2001) linked increasing fluoroquinolone resistance in *Campylobacter* directly to veterinary authorisation of enrofloxacin use in poultry production. The current susceptibility of Maiduguri isolates to ciprofloxacin and levofloxacin supports their continued use as first-line agents, but mandates ongoing surveillance given the trajectory of fluoroquinolone resistance globally.

Complete susceptibility to erythromycin (0% resistant; 67.3% sensitive) is a critical clinical finding, as macrolides specifically azithromycin are the first-line recommended treatment for severe human campylobacteriosis (Acheson & Allos, 2001). The detection of *erm(A)* by multiplex PCR in the absence of disc diffusion resistance is consistent with the documented role of the CmeABC efflux pump and sub-threshold 23S rRNA mutations in conferring latent macrolide resistance; this distinction is important because phenotypic susceptibility does not guarantee that the resistance genetic machinery is absent (Acheson & Allos, 2001).

The 57.1% MDR rate and the MAR index distribution (mean 0.40; IQR 0.30–0.40; maximum 0.70) unambiguously confirm that most *Campylobacter* isolates from Maiduguri's LBMs originate from high antibiotic-use environments. All MAR indices exceeded the 0.2 threshold established by Davis and Brown (2016) as indicative of such risk environments. The most frequently occurring resistance pattern NB–AMX–TET (14.3% of isolates; MAR=0.30) spans three antibiotic classes and recapitulates the antibiotics most used without prescription in Nigerian poultry. The dominance of patterns containing tetracycline (present in 11 of 13 identified patterns) reflects the ubiquity of tetracycline use and confirms this agent as the primary resistance driver in this setting, as noted in the broader Nigerian literature (Abd El-Hack et al., 2022).

For intermediate categories amoxicillin (85.7% intermediate) and rifampicin (81.6% intermediate) the data suggest that adjusted dosing might achieve clinical efficacy, but these agents should not be used empirically for campylobacteriosis without susceptibility confirmation (Sandhu et al., 2016). The 57.1% MDR rate is higher than rates reported in several comparable Sub-Saharan African LBM studies and underscores the urgent need for antibiotic stewardship programming, restriction of over-the-counter antimicrobial access in Nigerian poultry markets, and integration of routine *Campylobacter* AMR surveillance into existing National Action Plans on Antimicrobial Resistance.

Conclusions

This study provides the first comprehensive AMR characterisation of *Campylobacter* isolates from live bird markets in Maiduguri Metropolis, northeastern Nigeria. Tetracycline resistance (34.7%; 95% CI: 21.3%–49.4%), MDR prevalence (57.1%), elevated

MAR indices (mean 0.40; range 0.30–0.70), and molecular detection of *tet(A)* and *erm(A)* resistance genes together constitute a public health concern of high magnitude. The full susceptibility of all isolates to ciprofloxacin, levofloxacin, and erythromycin supports their continued use as empirical first-line therapy in this region. Immediate implementation of antibiotic stewardship policies, enforcement of veterinary prescription requirements, routine LBM surveillance, and farmer education on responsible antibiotic use are essential to contain MDR *Campylobacter* in northeastern Nigeria.

Abbreviations

AMR: Antimicrobial Resistance; MDR: Multidrug Resistance; MAR: Multiple Antibiotic Resistance; LBM: Live Bird Market; CLSI: Clinical and Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; PCR: Polymerase Chain Reaction; CI: Confidence Interval; IQR: Interquartile Range; NB: Norfloxacin; TET: Tetracycline; CH: Chloramphenicol; APX: Ampidox; AMX: Amoxicillin; RD: Rifampicin; CN: Gentamycin; E: Erythromycin; CPX: Ciprofloxacin; LEV: Levofloxacin.

Declarations

Ethics Approval and Consent to Participate

Ethical approval was obtained from the Borno State Ministry of Health. Informed consent was obtained from all human participants. All animal procedures complied with veterinary ethics guidelines.

Availability of Data and Materials

All datasets generated during this study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare no competing interests.

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Authors' Contributions

BUM, AM, and NNA designed the study, collected the data, and wrote the first draft of the manuscript. AM, NNA, ASS, and SGA oversaw the entire research process and critically reviewed the manuscript. BUM, FEO, IDK, and BJ contributed to data collation, data cleaning, and conducted the data analyses for the study. Finally, ML conducted the literature search and contributed to the writing of the final manuscript. All authors read and approved the final version of the manuscript.

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