JAC Antimicrob Resist
https://doi.org/10.1093/jacamr/dlae200

JAC-Antimicrobial Resistance

A nationwide survey of antimicrobial resistance of *Escherichia coli* isolated from broiler chickens in Malawi

Pilirani Chisembe [1], Masato Suzuki [2], Duc Trung Dao[1], Gilson Njunga[3], Joseph Nkhoma[3], Lecollins Mthilakuwili[3], Ryo Kinoshita-Daitoku[1], Eisuke Kuroda[1], Kouji Kimura[1] and Keigo Shibayama [1]*

[1]*Department of Bacteriology, Nagoya University Graduate School of Medicine, 65 Tsurumai, Showa, Nagoya, Aichi 466-8850, Japan;* [2]*Antimicrobial Resistance Research Center, National Institute of Infectious Diseases, 4-2-1 Aobacho, Higashimurayama, Tokyo 189-0002, Japan;* [3]*Department of Bacteriology, Central Veterinary Laboratory, Post Office Box 527, Lilongwe, Malawi*

*Corresponding author. E-mail: shibayama.keigo.i1@f.mail.nagoya-u.ac.jp

Received 29 August 2024; accepted 24 November 2024

Background: Antimicrobial resistance is a global health challenge with profound implications across sectors. Livestock, a significant field at the One Health interface, lacks sufficient information, particularly in low-resource settings such as Malawi.

Objectives: We determined the antimicrobial resistance rates of *Escherichia coli* isolated from broiler chickens in Malawi and explored the relationship between resistance genes across sectors using genomic analysis.

Methods: In 2023, we isolated 115 *E. coli* strains from 116 faecal and caecal samples from broiler chickens across Malawi. Antimicrobial susceptibility tests were performed using agar dilution method according to the Clinical Laboratory Standard Institute guidelines. Whole-genome sequencing was performed using Illumina sequencing.

Results: Notably, 50 isolates (44%) were resistant to cefotaxime. We detected ESBL $bla_{\text{CTX-M}}$ genes ($bla_{\text{CTX-M-55}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-65}}$, $bla_{\text{CTX-M-27}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-1}}$, and $bla_{\text{CTX-M-3}}$) in 48 cefotaxime-resistant isolates, which exhibited higher resistance rates to levofloxacin than non-ESBL-encoding isolates (29/48; 60% versus 20/67; 30%). All isolates were susceptible to colistin and carbapenems. High resistance rates were observed for tetracycline and co-trimoxazole commonly used in broiler chickens (90% and 70%, respectively). Sequence type 206 and phylogroup A were predominant (14% and 65%, respectively). In the genetic context of $bla_{\text{CTX-M}}$ genes, whole-genome alignment of the ESBL-producing isolates with reference plasmids from *E. coli* of various origins indicated significant similarity.

Conclusions: Antimicrobial resistance is highly prevalent among *E. coli* from broiler chickens in Malawi. Genomic analysis suggests potential transmission pathways for ESBL genes across sectors, necessitating further studies from One Health perspective.

Introduction

Antimicrobial resistance (AMR) is a pressing global health challenge. Its implications for humans, animals, and the environment are interconnected, necessitating the One Health approach. Surveillance, a One Health AMR strategy, is pivotal in providing essential data on emerging resistance patterns and trends.[1] Integrating surveillance data from all relevant sectors is crucial to obtaining a bird's-eye view of AMR development and dissemination across these sectors. Whole-genome analysis contributes to a deeper understanding of AMR epidemiology by revealing the relatedness among isolates. Performing this analysis is desirable to better understand AMR transmission between sectors. Identifying AMR genes in bacterial isolates is imperative, given their potential cross-species transfer.[2] It helps track the emergence and evolution of isolates and their AMR genes across sectors, which is the goal of integrating surveillance data from different sectors. However, surveillance and related

genome research activities in non-human sectors are rarely implemented, particularly in low-resource settings such as Malawi.

Livestock is a priority research area because of the widespread use of antimicrobials globally, accounting for 73% of all antimicrobials sold.[3] Intensive production systems, common in many countries, rely on routine antimicrobial use throughout livestock production.[3,4] Antimicrobial use is sometimes uncontrolled in the private sector and communities,[5,6] and such practices can contribute to developing multidrug resistance (MDR) pathogens in animals.[7] Broiler chickens, commonly consumed globally, are particularly significant at the One Health interface. *Escherichia coli* (*E. coli*), a commensal bacterium found in the intestinal microflora of animals, can indicate resistance levels in the bacterial population.[8]

ESBL-producing *E. coli* are increasingly prevalent globally, causing notable invasive diseases in Africa,[9] with emerging reports in broiler chickens across the continent. Cefotaxime (CTX)-hydrolyzing β-lactamase isolated in Munich ($bla_{\text{CTX-M}}$ genes) exhibits high resistance against a broad range of β-lactam antibiotics, including third-generation cephalosporins (3GCs).[10] Rapid spread through conjugative plasmid-mediated horizontal transfer and clonal spread has increased $bla_{\text{CTX-M}}$ prevalence worldwide,[11] including in broiler chickens.[12] The thriving poultry industry in Malawi has raised concerns about the potential cross-sector AMR inflow. However, information regarding *E. coli* AMR in livestock, particularly broiler chickens, is limited. Although a previous report indicated the emergence of MDR bacteria in livestock,[13] comprehensive data on the frequency rates and genome analyses are lacking. In this study, we conducted a nationwide survey of AMR *E. coli* in broiler chickens in Malawi for the first time, providing frequency rates and molecular epidemiology insights using antimicrobial susceptibility testing (AST) and whole-genome sequencing. This was to determine the resistance patterns of *E. coli* of broiler origin and explore the relationship between resistance genes across sectors.

Methods

Sample collection

In 2023, we collected 20 caecal and 96 faecal samples (116 samples) from apparently healthy broilers with consent from backyard farms (n = 72) and markets (n = 44) across three regions in Malawi (North, Mzuzu, and Nkhatabay; Central, Lilongwe, Dowa, Dedza, Ntcheu, Mchinji, and Kasungu; and South, Blantyre, and Zomba) (Figure 1). Intact caeca were collected from recently slaughtered birds at the market, packed in sterile sealable biohazard bags and transported at 4°C to the laboratory. Faecal samples were pooled from at least three fresh poops per crate or pen (in a smallholder farmer's house), packed and transported like the caecal samples.

Bacteria isolation

The caecal samples were inoculated onto two MacConkey agar (Eiken Chemical Co., Ltd, Japan) plates, with one supplemented with 4 mg/L CTX and the other without CTX (MacCTX+ and MacCTX−, respectively). The plates were incubated at 37°C for 18–24 h. The faecal samples were enriched in peptone water and incubated at 37°C for 18–24 h. These were inoculated on MacConkey agar plates (MacCTX+ and MacCTX−) and incubated under the same conditions. Red or pink colonies on MacCTX+ plates were inoculated into a Sulfur Indole Motility (SIM) medium (Sugiyama-Gen Co., Ltd, Japan) for identification and incubated at 37°C for 18–24 h. Lactose-fermenting isolates that tested positive for indole were presumed *E. coli* and stored at 4°C in a Casitone medium (Eiken Chemical Co., Ltd, Japan) for further analysis. If no red or pink colonies appeared on the MacCTX+ plates, the colonies from the MacCTX− plates were selected and processed in the same manner.

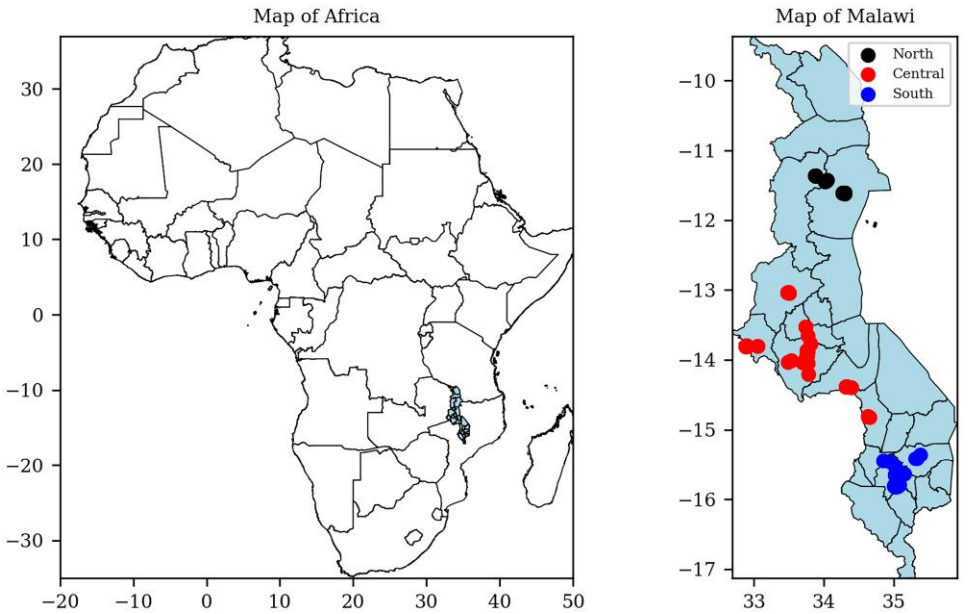

Figure 1. A map showing sample collection locations in Malawi. Malawi is highlighted on the map of Africa to the left. The map of Malawi, to the right, shows the locations of sample collection within 10 districts in three regions. These include the northern region (Mzuzu and Nkhatabay), central region (Dowa, Dedza, Kasungu, Lilongwe, Mchinji, and Ntcheu) and southern region (Blantyre and Zomba). The locations are marked in circles that are distinguished by region in the legend. Shapefiles used to generated the maps were extracted from ICPAC Geoportal (© OpenStreetMaps contributors) and GADM (© 2018-2022 GADM) for the map of Africa and Malawi, respectively.

Table 1. List of antimicrobials used for AST

Class	Antimicrobials
Penicillins	Ampicillin (AMP), amoxicillin (AMX), piperacillin (PIP)
β-Lactamase inhibitor combinations	Amoxicillin/clavulanate (AMC), piperacillin/tazobactam (TZP)
Cephalosporins	Cefepime (CPM), cefotaxime (CTX), ceftriaxone (CTR), ceftazidime (CAZ), cefmetazole (CMZ), cefazolin (CZ)
Monobactams	Aztreonam (AZT)
Carbapenems	Imipenem (IPM), meropenem (MEM)
Folate pathway inhibitor	Co-trimoxazole (COT)
Macrolide	Azithromycin (AZM)
Tetracyclines	Tetracycline (TET), doxycycline (DOXY), tigecycline (TGC), oxytetracycline (OTC)
Aminoglycosides	Gentamicin (GM), amikacin (AMK), streptomycin (SM), tobramycin (TOB), kanamycin (KM), neomycin
Quinolones	Ciprofloxacin (CIP), nalidixic acid (NAL), norfloxacin (NOR), levofloxacin (LVX)
Lipopeptides	Colistin (CL)
Phenicol	Chloramphenicol (CHL)
Nitrofuran	Nitrofurantoin (NIT)

Identification of isolates

Presumptive *E. coli* isolates were inoculated into SIM; Voges-Proskauer; Lysine, Indole, and Motility; Simmons Citrate; and Triple Sugar Iron media (Kyokuto Pharmaceutical Industries Co., Ltd, Japan) for confirmation. These were incubated at 37°C for 18–24 h, and the results were recorded after adding the necessary reagents. Confirmation was also performed using whole-genome analysis and the MALDI-TOF Mass Biotyper when the identification was indefinite. The confirmed *E. coli* isolates were stored in Tryptic Soy Broth (Difco™, USA) with glycerol 20% at −80°C for further testing.

Antimicrobial susceptibility test

AST was conducted using the agar dilution method, according to the Clinical Laboratory Standards Institute guidelines.[14] MIC was recorded after incubating Mueller–Hinton agar (Difco™, USA) plates at 37°C for 18–24 h. For colistin and tigecycline, broth microdilution was performed according to the European Committee on Antimicrobial Susceptibility Testing guidelines.[15] We tested 32 antibiotics for susceptibility (Table 1). MIC_{50} and MIC_{90} were calculated as the concentrations that inhibited 50% and 90% of the isolates, respectively. *E. coli* ATCC 25922 was used for quality control.

Whole-genome sequencing

DNA was extracted using the DNeasy Blood and Tissue Kit protocol (QIAGEN) and prepared using the Nextera XT DNA Library Prep Kit for Illumina sequencing (150 bp paired-end on HiseqX, Novaseq, and Miniseq platforms). Raw reads were trimmed with Trimmomatic, and *de novo* assembly was performed using Shovill version 1.1.0 (SPADES version 3.15.5) (https://github.com/tseemann/shovill). We checked the quality of the assembled sequences using dfast_QC (version 1.2.0).[16] Draft genomes of all *E. coli* strains were submitted to GenBank/DDBJ (BioProject number PRJDB18264). Genome assemblies were scanned against the ResFinder, PlasmidFinder, and PointFinder databases using the Staramr tool (version 0.10.0).[17] Phylogroups were detected using Clermon Typing (version 23.06) (http://clermontyping.iame-research.center/index.php),[18,19] whereas serotyping and quinolone resistance determining region (QRDR) mutations were identified using SerotypeFinder (version 2.0.1)[20] and ResFinder (version 4.5.0),[21,22] respectively. Whole-genome alignment was performed using the QIAGEN CLC workbench (version 24.0.1) (https://digitalinsights.qiagen.com/).

The search and comparison of reference plasmids carrying $bla_{CTX\text{-}M}$ *genes*

Direct search for plasmids on the National Centre for Biotechnology Information (NCBI) nucleotide database was performed using gene name, plasmid, and *E. coli* (for example, $bla_{CTX\text{-}M\text{-}27}$[All Field] AND ("unidentified plasmid" [Organism] OR plasmid [All Fields] AND ("*Escherichia coli*" [Organism] OR *E. coli* [All Fields]) as search words (https://www.ncbi.nlm.nih.gov/). The plasmids were selected based on fully assembled sequences from long-read sequencing, a unique source or host (human, environment, or animal), and *E. coli* species. These plasmids were aligned to draft genomes carrying specific $bla_{CTX\text{-}M}$ genes using the whole-genome alignment tool on the CLC workbench. Replicon types were obtained from the NCBI, and those lacking information were analysed using the MOB-Typer tool (version 3.0.3)[23] on Galaxy Europe (https://usegalaxy.eu/). Table S4 (available as Supplementary data at *JAC-AMR* Online) shows reference genome information.

Data analysis

Data were cleaned and managed using Microsoft Excel (version 2310). Graphs and maps were generated using the Python environment in Deepnote (https://deepnote.com/). The shapefiles for the Malawi and Africa maps were sourced from the Global Administrative Areas (https://gadm.org/data.html) and the IGAD Climate Prediction and Application Centre geoportal (https://geoportal.icpac.net/layers/geonode:afr_g2014_2013_0/layer_export#/), respectively. Descriptive statistics were performed using IBM SPSS Statistics (version 29.0.1.0(171)), and differences were analysed using Pearson's χ^2 test (Fisher's exact test, where appropriate) with a significance level of 0.05. Data were compared across regions using the Kruskal–Wallis test with Bonferroni-adjusted *P* values.

Results

Isolate characteristics

We obtained 115 *E. coli* isolates from 116 faecal and caecal samples (one isolate per sample). Of these, 50 (43.5%) were obtained from MacCTX+ plates and 65 (56.5%) from MacCTX− plates. The *E. coli* isolates belonged to 49 different sequence types (including ST206 and ST48, with frequencies of >5%) and belonged to the phylogroups A, B1, B2, C, D, E, and G (Table S1). None of the *E. coli* isolates belonged to ST131 or O157:H7 serotypes.

Table 2. Minimum inhibitory concentration distribution for β-lactam in CTX-resistant *E. coli* isolates ($n=50$)

	Concentration (mg/L)														
Agent	<0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128	MIC_{50}[a]	MIC_{90}[b]
Imipenem		2	40	8										0.25	0.5
Meropenem	50													<0.06	<0.06
Aztreonam			1					3	**6**	**12**	**11**	**14**	**3**	64	128
Cefepime	1						1	4	**4**	**3**	**5**	**5**	**27**	>128	>128
Ceftriaxone										**1**			**49**	>128	>128
Cefotaxime										**1**		**1**	**48**	>128	>128
Ceftazidime				2	3	3	4	4	**8**	**10**	**10**	**4**	**2**	64	128
Cefmetazole					4	17	24	4	1					4	4
Cefazolin							1						**49**	>128	>128
TZP				1	12	28	9							2/4	4/4
Piperacillin						1							**49**	>128	>128
AMC					7	11	23	8	1					4/2	8/4
Amoxicillin							1						**49**	>128	>128
Ampicillin								1					**49**	>128	>128

All numbers in bold against each agent represent resistant isolates.
TZP, piperacillin/tazobactam; AMC, amoxicillin/clavulanate.
[a]MIC where 50% of the *E. coli* isolates were inhibited.
[b]MIC where 90% of the *E. coli* isolates were inhibited.

Table 3. Minimum inhibitory concentration distribution for β-lactam in CTX-susceptible *E. coli* isolates ($n=65$)

	Concentration (mg/L)													MIC_{50}[a]	MIC_{90}[b]
Agent	<0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128		
Imipenem		5	56	4										0.25	0.25
Meropenem	65													<0.06	<0.06
Aztreonam	2	23	23	8		1		1	**1**	**1**	**4**	**1**		0.25	16
Cefepime	43	18	2			1	1							<0.06	0.125
Ceftriaxone	41	22	1	1										<0.06	0.125
Cefotaxime	9	44	10	2										0.125	0.25
Ceftazidime			10	36	16		2	1						0.5	1
Cefmetazole					25	27	12		1					2	4
Cefazolin						21	24	8	9		**1**		**2**	4	16
TZP				1	20	35	9							2/4	4/4
Piperacillin					3	25	11		1	1			**24**	4	>128
AMC				7	12	18	21	5	2					2/1	4/2
Amoxicillin						5	21	4		1			**34**	>128	>128
Ampicillin						1	14	18	2			**2**	**28**	8	>128

All numbers in bold against each agent represent resistant isolates.
TZP, piperacillin/tazobactam; AMC, amoxicillin/clavulanate.
[a]MIC where 50% of the *E. coli* isolates were inhibited.
[b]MIC where 90% of the *E. coli* isolates were inhibited.

Antimicrobial susceptibility profiles

We determined the MIC distributions, including the MIC_{50} and MIC_{90} values (Tables 2–5) and antibiograms (Figure 2) stratified by susceptibility to CTX. Of the 115 *E. coli* isolates, 50 (43.5%) were resistant to CTX (MIC ≥ 4 mg/L), indicating that 43.5% of broilers carried CTX-resistant *E. coli*. All CTX-resistant isolates were from MacCTX+ plates while susceptible ones (65, 56.5%) were from the MacCTX− plates. The rates of resistance to ceftriaxone and ceftazidime, both 3GCs, were 43.5% and 29.6% (34/115), respectively, and all resistant strains were CTX resistant. The same observation was made with the other cephalosporins except for cefazolin where 4.6% (3/65) CTX-susceptible isolates

Table 4. Minimum inhibitory concentration distribution for non-β-lactam antibiotics in CTX-resistant *E. coli* isolates ($n=50$)

	Concentration, mg/L													MIC_{50}^{a}	MIC_{90}^{b}
Agent	<0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128		
Tigecycline		14	35	1										0.25	0.25
Colistin		44	6											0.125	0.25
Nitrofurantoin									9	34	5	**2**		32	64
Azithromycin							5	17	15	**1**	**5**	**2**	**5**	16	128
Chloramphenicol							1	5	14		**3**	**6**	**21**	128	>128
Amikacin							28	18	2	1			**1**	4	8
Kanamycin							4	25	9	4	**1**		**7**	8	>128
Tobramycin					1	23	7	2	**4**	**5**	**1**	**3**	**4**	4	128
Gentamicin					10	20					**5**	**2**	**13**	2	>128
Streptomycin							1	8	7	1	4	6	23	128	>128
Neomycin						8	22	10	4		1	1	4	4	64
Ciprofloxacin	3	1	1	11	5			**3**	**9**	**8**	**5**	**2**	**2**	16	64
Levofloxacin	2	2		4	10	2		**2**	**10**	**12**	**1**	**2**	**3**	16	64
Norfloxacin	1	2	1		10	6			**2**	**1**	**10**	**3**	**14**	64	>128
Nalidixic acid						1	1	2	3	**2**			**41**	>128	>128
Co-trimoxazole	9		5	2				**34**						>8/152	>8/152
Doxycycline						1	2	1	**5**	**25**	**13**	**3**		32	64
Oxytetracycline							1	2					**47**	>128	>128
Tetracycline						2	1				**1**	**17**	**29**	>128	>128

All numbers in bold against each agent represent resistant isolates.
[a]MIC where 50% of the *E. coli* isolates were inhibited.
[b]MIC where 90% of the *E. coli* isolates were inhibited.

Table 5. Minimum inhibitory concentration distribution for non-β-lactam antibiotics in CTX-susceptible *E. coli* isolates ($n=65$)

	Concentration, mg/L													MIC_{50}^{a}	MIC_{90}^{b}
Agent	<0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128		
Tigecycline	2	14	47	3										0.25	0.25
Colistin		61	3	1										0.125	0.125
Nitrofurantoin									10	44	**9**	**2**		32	64
Azithromycin							7	35	20	**2**			**1**	8	16
Chloramphenicol								21	33	**3**	**1**	**3**	**4**	16	128
Amikacin						2	38	23	2					4	8
Kanamycin							9	36	7	4			**9**	8	>128
Tobramycin					11	33	6		**5**	**3**	**4**	**1**	**2**	2	32
Gentamicin					5	43	4					**1**	**12**	2	>128
Streptomycin							2	19	13	6	5	6	14	16	>128
Neomycin						19	29	8	3			4	2	4	16
Ciprofloxacin	24		3	13	10	2	**1**		**3**	**6**	**3**			0.5	32
levofloxacin	19	2		5	14	5			**3**	**7**	**1**	**4**	**5**	1	128
Norfloxacin	12	7	2	2	6	16		1	**1**	**5**	**3**	**2**	**8**	2	128
Nalidixic acid							12	15	5	**7**	**4**		**22**	32	>128
Co-trimoxazole	16		2		1		**3**	**43**						>8/152	>8/152
Doxycycline					2	3	8	1	**17**	**21**	**11**	**2**		32	64
Oxytetracycline							6	5	**1**				**53**	>128	>128
Tetracycline					3	9			**1**		**2**	**21**	**29**	128	>128

All numbers in bold against each agent represent resistant isolates.
[a]MIC where 50% of the *E. coli* isolates were inhibited.
[b]MIC where 90% of the *E. coli* isolates were inhibited.

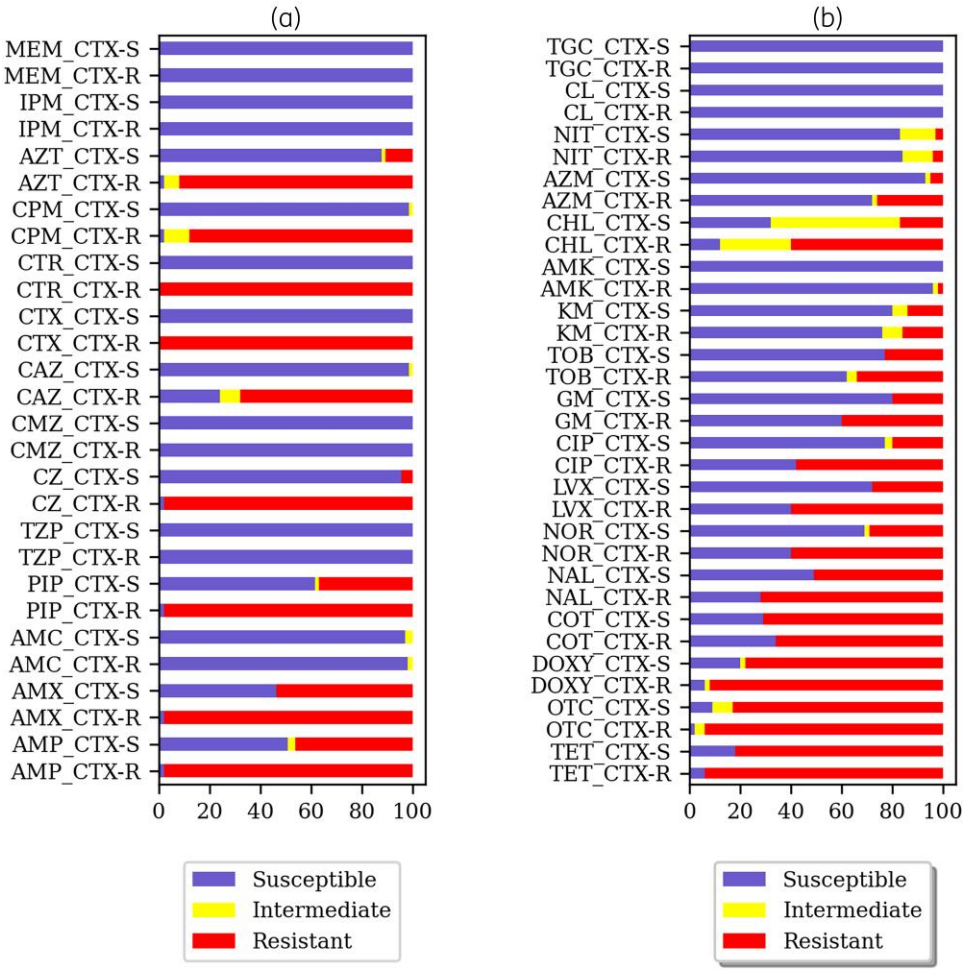

Figure 2. Antimicrobial susceptibility Antibiogram for *E. coli* isolates. (a) illustrates susceptibility against β-lactams and (b) against non-β-lactams stratified by CTX susceptibility. The levels of susceptibility (susceptible, intermediate, and resistant) are indicated in the legend. MEM, meropenem; IPM, imipenem; AZT, aztreonam; CPM, cefepime; CTR, ceftriaxone; CTX, cefotaxime; CAZ, ceftazidime; CMZ, cefmetazole; CZ, cefazolin; TZP, piperacillin/tazobactam; PIPC, piperacillin; AMC, amoxicillin/clavulanate; AMX, amoxicillin; AMP, ampicillin; CTX-S, cefotaxime-susceptible; CTX-R, cefotaxime-resistant; TGC, tigecycline; CL, colistin; NIT, nitrofurantoin; AZM, azithromycin; CHL, chloramphenicol; AMK, amikacin; KM, kanamycin; TOB, tobramycin; GM, gentamicin; CIP, ciprofloxacin; LVX, levofloxacin; NOR, norfloxacin; NAL, nalidixic acid; COT, co-trimoxazole; DOXY, doxycycline; OTC, oxytetracycline; TET, tetracycline.

were resistant (MIC ≥ 32 mg/L) (Table 3). The CTX-susceptible isolates exhibited resistance against other β-lactams, albeit in lower levels than CTX-resistant isolates [amoxicillin (52.3% versus 98%), ampicillin (46.2% versus 98%), piperacillin (36.9% versus 98%), and aztreonam (10.8% versus 92%)] (Figure 2a). The MIC_{90} of penicillins with β-lactamase inhibitors (amoxicillin/clavulanate and piperacillin/tazobactam) fell into the criteria of susceptible (MIC ≤ 8/4 and ≤16/4 mg/L, respectively) (Tables 2 and 3), suggesting that ESBL most caused resistance to 3GCs. Resistance to carbapenems was not detected.

The MIC values and antibiogram of antimicrobials other than β-lactams are presented in Tables 4 and 5 and Figure 2(b), respectively. We observed high resistance rates in all *E. coli* isolates to tetracyclines (90.4%, 104/115), co-trimoxazole (69.6%, 80/115), and quinolones (66.1%, 76/115). CTX-resistant isolates showed higher resistance to many antimicrobials, especially quinolones and gentamicin ($P < 0.05$), and no significant differences were observed for tetracyclines, co-trimoxazole, or nitrofuran. Overall, 95% of the *E. coli* isolates were classified as MDR, as they were resistant to at least three antimicrobial classes. ESBL-producing isolates (CTX resistant), being more likely to be MDR, exhibited higher rates than non-ESBL-producing isolates (CTX susceptible) (100% and 91%, respectively). All isolates were susceptible to colistin and tigecycline.

Table S2 shows the geographical distribution of resistance. For several antibiotics, including oxytetracycline, cefepime, ceftriaxone, and ampicillin, the resistance rates of *E. coli* isolates varied significantly by region ($P < 0.05$). However, the resistance rates of these antibiotics between the central and northern regions did not differ significantly. In contrast, the southern region generally exhibited lower resistance rates to oxytetracycline, cephalosporins, and ampicillin than the central and northern regions. In

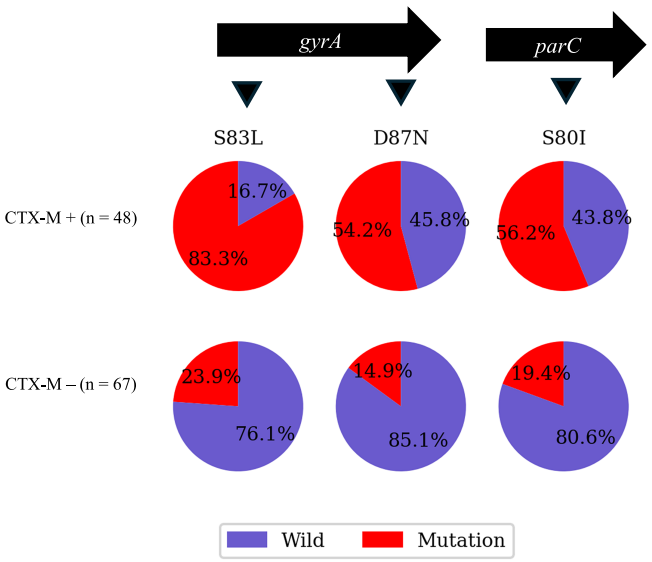

Figure 3. The distribution of QRDR mutations among *E. coli* isolates stratified by the presence of bla_{CTX-M} genes. The QRDR mutations include S83L and D87N in *gyrA* gene (where serine was replaced by leucine at position 83 while aspartic acid by asparagine at position 87, respectively) and S80I in *parC* gene (where serine was replaced by isoleucine at position 80). The presence and absence of the mutations is distinguished in the legend, with "wild" referring to wild-type sequences for isolates without QRDR mutations.

addition, the southern region exhibited the lowest MDR (84%) compared to other regions (98.5% and 95.7% for the central and northern regions, respectively) ($P < 0.05$).

Distribution of β-lactamase-encoding genes

Of the 50 CTX-resistant isolates, 48 were ESBL producers carrying bla_{CTX-M} ESBL genes. We detected only the CTX-M-1 and CTX-M-9 groups, including $bla_{CTX-M-55}$ ($n = 21$), $bla_{CTX-M-1}$ ($n = 1$), $bla_{CTX-M-3}$ ($n = 2$), and $bla_{CTX-M-15}$ ($n = 1$) in the CTX-M-1 group and $bla_{CTX-M-14}$ ($n = 9$), $bla_{CTX-M-65}$ ($n = 7$), and $bla_{CTX-M-27}$ ($n = 7$) in the CTX-M-9 group. The most prevalent gene was $bla_{CTX-M-55}$ (43.8%, 21/48), followed by $bla_{CTX-M-14}$ (18.8%, 9/48). ResFinder did not detect the responsible resistance gene in the remaining two CTX-resistant isolates. A class D β-lactamase bla_{OXA-10} (an ESBL enzyme that could confer resistance to 3GCs) was detected in two CTX-susceptible isolates. It might not have been produced at high levels in the CTX-susceptible isolates for expression. Of the 65 CTX-susceptible isolates, 24 carried non-ESBL bla_{TEM} genes, 2 carried bla_{LAP-2} genes, and 1 carried bla_{CARB-2} (Table S3). These isolates were resistant to ampicillin, amoxicillin, piperacillin, and cefazolin, except four isolates carrying $bla_{TEM-135}$, bla_{TEM-1C}, bla_{TEM-36}, and $bla_{TEM-214}$.

Prevalence of AMR mechanisms other than β-lactamase

Apart from the β-lactamase genes, we detected various AMR genes ($n = 68$). The tetracycline resistance gene *tet(A)* was highly prevalent: 92.0% (46/50) and 81.5% (53/65) in CTX-resistant and CTX-susceptible *E. coli* isolates, respectively (Table S3). Resistance genes for aminoglycosides (16/68, 23.5%) and folate pathway inhibitors (10/68, 14.7%) were predominant. QRDR mutations including S83L ($n = 56/115$) and D87N ($n = 36/115$) in the *gyrA* gene and S80I ($n = 40/115$) in the *parC* gene were also detected. All 44 levofloxacin-resistant isolates harboured at least one QRDR mutation. When the QRDR mutations were stratified by bla_{CTX-M} genes, 83.3% (40/48) of the isolates carrying bla_{CTX-M} genes had mutations. In contrast, isolates with no bla_{CTX-M} genes had fewer mutations (16/67, 23.9%) (Figure 3).

Comparison of draft genome sequences with the complete reference plasmids harbouring bla_{CTX-M} *genes*

bla_{CTX-M} genes are typically carried on plasmids. We aligned the draft genome sequences of *E. coli* isolates harbouring bla_{CTX-M} genes against various complete reference plasmid sequences of *E. coli* strains of various origins (one plasmid per source or host; animal, humans, and the environment). Notably, several reference plasmids aligned well with the genome sequences obtained in this study (Figures 4 and 5). The percentage of the total aligned sequence length (*x*-axis) was <50%, but the sequence identity (*y*-axis) of the aligned sequences was typically >94% (Table S5), indicating a high degree of nucleotide similarity in the aligned regions. This further suggests that the isolates harbouring the bla_{CTX-M} genes carried it on a plasmid that was phylogenetically related to the reference plasmids, despite the different host origins (Figures 4 and 5). The aligned regions included mobile genetic element parts, such as *ISEcp1*, *IS5*, *IS26*, *tn3*, and *int*, along with other conjugative plasmid structures (*traC*, *traD*, *traY*, *hok*, *doc*, and *pemK*). Other antibiotic-resistant genes (ARGs), such as bla_{TEM}, *tet(A)*, *sul2*, and *aac(3)-IIa* were also present, in addition to bla_{CTX-M} genes (Figures S2–S8). The highest observed alignment percentage was 47.56% for an isolate harbouring $bla_{CTX-M-1}$ (*E. coli* strain KU15F68, accession number: BAAFOL010000001-BAAFOL010000144) against *pN603* (accession number: LC567083) of human origin from Japan (Figure 4). In this study, the alignment percentage, sequence identity, and source of origin showed no consistent relationship, suggesting that the host factor did not significantly impact the alignment results (Figure S1).

Discussion

To the best of our knowledge, this is the first study documenting the resistance of *E. coli* isolated from broiler chickens in Malawi through standardized AST and whole-genome sequencing analysis. This study marks a crucial step towards implementing a One Health approach to combat AMR in the country, aiding policymakers and researchers in devising effective mitigation strategies. Using phenotypic and genotypic approaches, we provided a comprehensive AMR picture, particularly CTX resistance. Our findings revealed notable resistance to tetracyclines (90%), β-lactams (78%), co-trimoxazole (70%), and quinolones (66%). These resistance patterns could be partially attributed to the extensive use of antimicrobials in Malawian livestock farming,[24] with tetracyclines, procaine penicillin G, streptomycin, and flumequine commonly dispensed by agrovet shops,[4,6] and frequently used as growth promoters in daily feed,[6] thereby enhancing AMR selection and spread. Cephalosporin use in livestock is undocumented in Malawi; we found a high resistance rate to

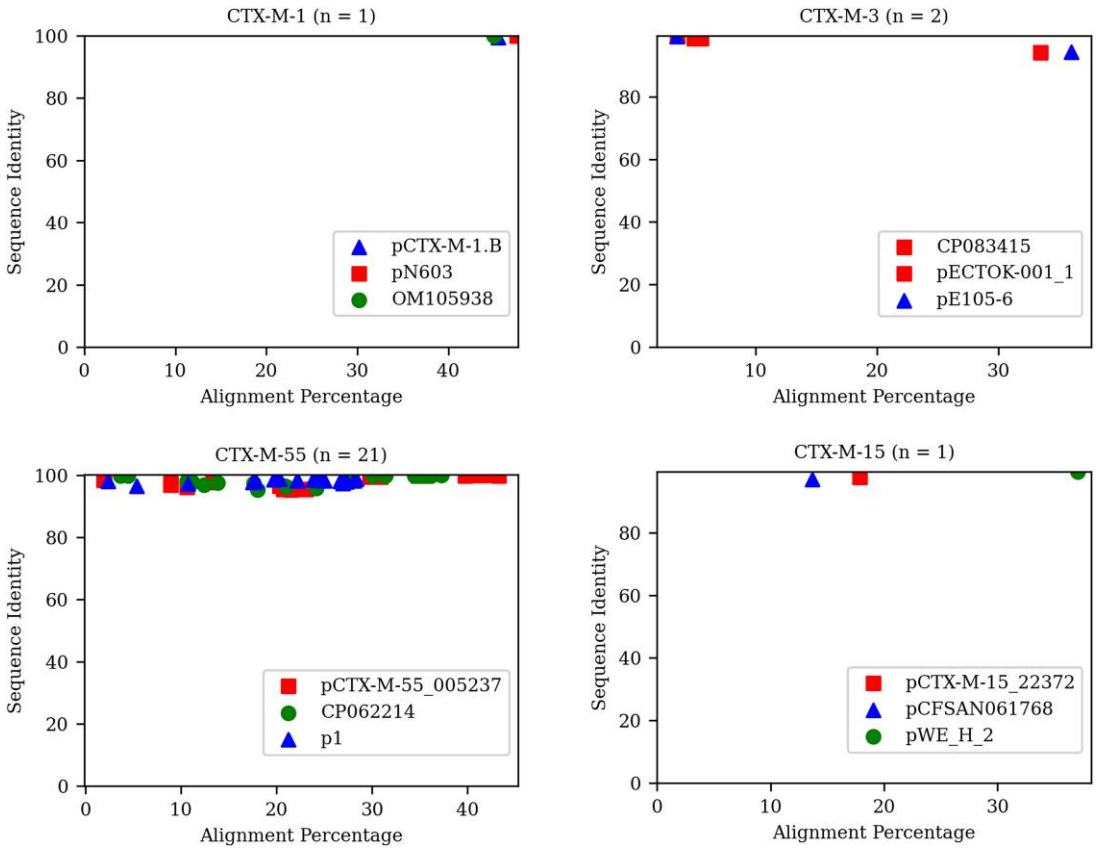

Figure 4. Alignment of plasmids harbouring CTX-M-1 type genes. The *x*- and *y*-axes are the alignment and sequence identity percentages, respectively. n is the total number of isolates harbouring the specific $bla_{\text{CTX-M}}$ gene. Reference plasmids are distinguished in the legend by symbols: squares represent reference plasmids of *E. coli* strains isolated from humans, triangles represent those from animals (pig, turkey, and fish), and circles represent those from the environment (wastewater and river water).

3GCs (44.3%, $n=51/115$), suggesting the existence of a driver of 3GC resistance. This observation is of great concern because 3GCs are the last-line antibiotics frequently used in intensive care units in Malawi.[25] The resistance rate against cefepime was 38.3%, which was higher than that in the USA (16.83%)[26] but lower than that in Egypt (95.8%).[27] These results indicate the threat of treating ESBL-producing bacterial infections due to rising resistance. Reports on 3GC use during *in ovo* vaccination of newly hatched chicks in the poultry industry have been documented in several countries, where it was associated with increased resistance.[28–31] However, some papers reported that antimicrobial use may not always be related to the prevalence of ESBL-producing *E. coli* in poultry,[26,32] indicating these pathogens' complexity. An extensive investigation of 3GC use throughout broiler chicken production is necessary to identify and understand the drivers of resistance in Malawi.

Of the ESBL-encoding genes, $bla_{\text{CTX-M}}$ is most frequently detected in *E. coli* isolates of human origin.[11] It has also been detected in *E. coli* of poultry origin, including broiler chickens.[26,33,34] We detected $bla_{\text{CTX-M}}$ ESBL-encoding genes only, specifically $bla_{\text{CTX-M-1}}$ ($n=1$), $bla_{\text{CTX-M-3}}$ ($n=2$), $bla_{\text{CTX-M-14}}$ ($n=10$), $bla_{\text{CTX-M-15}}$ ($n=1$), $bla_{\text{CTX-M-27}}$ ($n=7$), $bla_{\text{CTX-M-55}}$ ($n=21$), and $bla_{\text{CTX-M-65}}$ ($n=7$). This may have been due to the selective pressure from CTX as $bla_{\text{CTX-M}}$ exhibits better hydrolytic activity against CTX than other ESBLs. These types have previously been detected in humans worldwide,[11] and $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-27}}$ have specifically been reported in Malawi.[9,35,36] The presence of $bla_{\text{CTX-M}}$ suggests transmission between broilers and humans, possibly facilitated by horizontal gene transfer (HGT) involving mobile genetic elements. ARG transmission may occur through direct contact with chickens or consumption of chicken products, exposing individuals to drug-resistant bacteria. Sequence alignment analysis indicated highly conserved regions of similarity between the reference plasmids and draft genomes at the nucleotide level, including the $bla_{\text{CTX-M}}$ genes, suggesting ongoing transmission between populations. The presence of mobile genetic elements (such as *ISEcp1*, *tn3*, and *int*) and some conjugative plasmid's structures (such as *traC*, *hok*, and *pemK*) in the aligned regions suggests that HGT plays a crucial role in the disseminating $bla_{\text{CTX-M}}$ genes and other ARGs. These mobile genetic elements are associated with $bla_{\text{CTX-M}}$ gene mobilization,[12] indicating a mechanism to transfer these genes across sectors. ESBL production is often associated with fluoroquinolone resistance. In humans, ST131 is one of major pathogenic lineages showing such profile. Although ST131 was not isolated in this study, most $bla_{\text{CTX-M}}$-carrying isolates were fluoroquinolone resistant with QRDR mutations, further indicating this link across various *E. coli* STs. The co-occurrence of ESBL-encoding genes

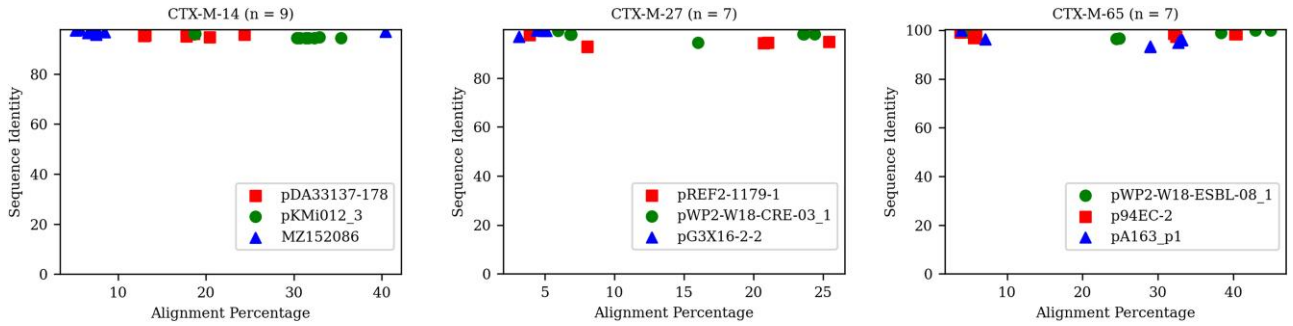

Figure 5. Alignment of plasmids harbouring CTX-M-9 type genes. The *x*- and *y*-axes are the alignment and sequence identity percentages, respectively. n is the total number of isolates harbouring the specific bla_{CTX-M} gene. Reference plasmids are distinguished in the legend by symbols: squares represent reference plasmids of *E. coli* strains isolated from humans, triangles represent those from animals (pig and bovine), and circles represent those from the environment (wastewater and river water).

and QRDR mutations suggests that MDR phenotypes can be acquired through both mobile genetic elements and chromosomal point mutations, restricting treatment options for infections caused by these strains. Clustering of ARGs, such as bla_{TEM}, *tet(A)*, and *sul2*, within close genetic regions suggest co-selection, where using one antibiotic can select for resistance to multiple others. This study showed a 95% prevalence of MDR *E. coli* isolates, which is consistent with reports from other African countries [Ghana (95.8%),[37] Nigeria (94.6%),[38] and Tanzania (86.76%)[39]]. In this study, none of the isolates were resistant to colistin or carried *mcr* genes, despite the common use in Malawi, highlighting the need to prevent the spread of colistin resistance.

The low extent of alignment (<50%) suggests that plasmids carrying bla_{CTX-M} genes diverged significantly regarding their overall structure and organization because of genomic rearrangements during long-term propagation and transmission. The lack of a consistent relationship between the alignment percentage, sequence identity, and source of origin (Figure S1) indicates that the relatedness of plasmids carrying bla_{CTX-M} genes is a significant factor in understanding resistance transmission among various hosts.[40] The reference plasmids originated from diverse geographical locations (Figure S9), implying that these genes circulate not only across different sectors but also globally. To obtain more comprehensive perspective, we will create a complete genome sequence and do overall comparison at a next step. A step towards achieving the goals of the One Health approach is to enhance the knowledge of cross-host transmission by understanding the specific genetic elements and mechanisms driving the observed similarities and differences through further research.[41]

Conclusions

To the best of our knowledge, this is the first study to reveal a concerning prevalence of AMR among *E. coli* isolates from broilers in Malawi. Rates of resistance to commonly used antimicrobials were typically high. Furthermore, cephalosporins were not included in the feed, but the rate of resistance to CTX was high, highlighting the significant challenges AMR poses and its complexity. Effective mitigation of AMR can be achieved through antimicrobial stewardship campaigns. Resistance genes, including ESBL-encoding genes, can spread to bacteria, which can infect humans through mobile gene elements. Our genomic analysis suggests potential transmission pathways for ESBL-encoding genes across different sectors. Comprehensive research on the dynamics of AMR dissemination across host populations is required to address the associated risks. From a One Health perspective, further large-scale surveys, genome analyses, and bioinformatics studies are needed to reveal the transmission routes of resistance genes between humans and other sectors. Such scientific evidence can help assess risks, identify targets for countermeasures, and develop effective policies.

Acknowledgements

We sincerely appreciate all the farmers and vendors for participating in this study. Special thanks to the Department of Animal Health and Livestock Development and the Central Veterinary Laboratory (CVL) for their invaluable support. We are grateful to Mary Dulira, Martha Munthali, and the CVL staff for their assistance with sample collection. We also thank Hiromi Ajisaka for technical support during AST and Illumina sequencing and to the Bacteriology Department at Nagoya University.

Funding

This study was supported by the Japan Agency for Medical Research and Development (grant numbers JP23fk0108604, JP24fk0108665, JP24fk0108683, JP24fk0108712, 24fk0108642, JP24gm1610003, JP24wm0225029, and JP24wm0225022) and Japan Society for the Promotion of Science KAKENHI (grant number 23KK0151).

Transparency declarations

None to declare.

Ethics statement

Ethical approval was obtained from the Department of Animal Health and Livestock Development of the Ministry of Agriculture in Malawi (ref. DAHLD/AHC/01/2023/7). All protocols were performed according to the approved ethical terms and conditions. The ARRIVE guidelines were not applicable.

Supplementary data

Tables S1–S5 and Figures S1–S9 are available as Supplementary data at *JAC-AMR* Online.

References

1 McEwen SA, Collignon PJ. Antimicrobial resistance: a one health perspective. *Microbiol Spectr* 2018; **6**. https://doi.org/10.1128/microbiolspec.ARBA-0009-2017

2 Moser KA, Zhang L, Spicknall I *et al*. The role of mobile genetic elements in the spread of antimicrobial-resistant *Escherichia coli* from chickens to humans in small-scale production poultry operations in rural Ecuador. *Am J Epidemiol* 2018; **187**: 558–67. https://doi.org/10.1093/aje/kwx286

3 Van Boeckel TP, Pires J, Silvester R *et al*. Global trends in antimicrobial resistance in animals in low- and middle-income countries. *Science* 2019; **365**: eaaw1944. https://doi.org/10.1126/science.aaw1944

4 Mankhomwa J, Tolhurst R, M'biya E *et al*. A qualitative study of antibiotic use practices in intensive small-scale farming in urban and peri-urban Blantyre, Malawi: implications for antimicrobial resistance. *Front Vet Sci* 2022; **9**: 876513. https://doi.org/10.3389/FVETS.2022.876513

5 Makoka MH, Miller WC, Hoffman IF *et al*. Bacterial infections in Lilongwe, Malawi: aetiology and antibiotic resistance. *BMC Infect Dis* 2012; **12**: 67. https://doi.org/10.1186/1471-2334-12-67

6 Kainga H, Phonera MC, Chikowe I *et al*. Determinants of knowledge, attitude, and practices of veterinary drug dispensers toward antimicrobial use and resistance in main cities of Malawi: a concern on antibiotic stewardship. *Antibiotics (Basel)* 2023; **12**: 149. https://doi.org/10.3390/antibiotics12010149

7 Aarestrup FM, Kruse H, Tast E *et al*. Associations between the use of antimicrobial agents for growth promotion and the occurrence of resistance among *Enterococcus faecium* from broilers and pigs in Denmark, Finland, and Norway. *Microb Drug Resist* 2000; **6**: 63–70. https://doi.org/10.1089/MDR.2000.6.63

8 Persoons D, Bollaerts K, Smet A *et al*. The importance of sample size in the determination of a flock-level antimicrobial resistance profile for *Escherichia coli* in broilers. *Microb drug Resist* 2011; **17**: 513–9. https://doi.org/10.1089/mdr.2011.0048

9 Tegha G, Ciccone EJ, Krysiak R *et al*. Genomic epidemiology of *Escherichia coli* isolates from a tertiary referral centre in Lilongwe, Malawi. *Microb Genom* 2021; **7**: mgen000490. https://doi.org/10.1099/MGEN.0.000490

10 Zhao WH, Hu ZQ. Epidemiology and genetics of CTX-M extended-spectrum β-lactamases in Gram-negative bacteria. *Crit Rev Microbiol* 2013; **39**: 79–101. https://doi.org/10.3109/1040841X.2012.691460

11 Bevan ER, Jones AM, Hawkey PM. Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. *J Antimicrob Chemother* 2017; **72**: 2145–55. https://doi.org/10.1093/JAC/DKX146

12 D'Andrea MM, Arena F, Pallecchi L *et al*. CTX-M-type β-lactamases: a successful story of antibiotic resistance. *Int J Med Microbiol* 2013; **303**: 305–17. https://doi.org/10.1016/J.IJMM.2013.02.008

13 Kalumbi MH, Katuah ZJ, Nyirenda AE *et al*. Prevalence of multidrug-resistant bacterial isolates from chickens, goats, cattle and pigs in Bvumbwe, Malawi. *Appli Microbiol* 2022; **8**: 234. https://doi.org/10.35284/2471-9315.22.8.234

14 Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing*. 26th informational supplement. CLSI, 2016. CLSI Document M100-S26.

15 The European Committee on Antimicrobial Susceptibility Testing. *Breakpoint Tables for Interpretation of MICs and Zone Diameters*. Version 13.0, 2023. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_13.0_Breakpoint_Tables.pdf

16 Tanizawa Y, Fujisawa T, Nakamura Y. DFAST: a flexible prokaryotic genome annotation pipeline for faster genome publication. *Bioinformatics* 2018; **34**: 1037–9. https://doi.org/10.1093/bioinformatics/btx713

17 Bharat A, Petkau A, Avery BP *et al*. Correlation between phenotypic and in silico detection of antimicrobial resistance in *Salmonella enterica* in Canada using Staramr. *Microorganisms* 2022; **10**: 292. https://doi.org/10.3390/microorganisms10020292

18 Beghain J, Bridier-Nahmias A, Nagard HL *et al*. ClermonTyping: an easy-to-use and accurate in silico method for *Escherichia* genus strain phylotyping. *Microb Genom* 2018; **4**: e000192. https://doi.org/10.1099/mgen.0.000192

19 Clermont O, Dixit OVA, Vangchhia B *et al*. Characterization and rapid identification of phylogroup G in *Escherichia coli*, a lineage with high virulence and antibiotic resistance potential. *Environ Microbiol* 2019; **21**: 3107–17. https://doi.org/10.1111/1462-2920.14713

20 Joensen KG, Tetzschner AMM, Iguchi A *et al*. Rapid and easy in silico serotyping of *Escherichia coli* isolates by use of whole-genome sequencing data. *J Clin Microbiol* 2015; **53**: 2410–26. https://doi.org/10.1128/JCM.00008-15

21 Bortolaia V, Kaas RS, Ruppe E *et al*. ResFinder 4.0 for predictions of phenotypes from genotypes. *J Antimicrob Chemother* 2020; **75**: 3491–500. https://doi.org/10.1093/jac/dkaa345

22 Camacho C, Coulouris G, Avagyan V *et al*. BLAST+: architecture and applications. *BMC Bioinformatics* 2009; **10**: 421. https://doi.org/10.1186/1471-2105-10-421

23 Robertson J, Nash JHE. MOB-suite: software tools for clustering, reconstruction and typing of plasmids from draft assemblies. *Microb Genom* 2018; **4**: e000206. https://doi.org/10.1099/mgen.0.000206

24 Ngunguni SM, Moodley A, Msefula C *et al*. Patterns and drivers of antibiotic use in small-scale broiler production systems in Lilongwe District, Malawi. *Prev Vet Med* 2024; **230**: 106263. https://doi.org/10.1016/J.PREVETMED.2024.106263

25 Kayambankadzanja RK, Lihaka M, Barratt-Due A *et al*. The use of antibiotics in the intensive care unit of a tertiary hospital in Malawi. *BMC Infect Dis* 2020; **20**: 776. https://doi.org/10.1186/S12879-020-05505-6

26 Parzygnat JL, Crespo R, Koci MD *et al*. Widespread prevalence of plasmid-mediated blaCTX-M type extended-spectrum beta-lactamase *Escherichia coli* in backyard broiler production systems in the United States. *PLoS One* 2024; **19**: e0304599. https://doi.org/10.1371/journal.pone.0304599

27 Younis G, Awad A, Mohamed N. Phenotypic and genotypic characterization of antimicrobial susceptibility of avian pathogenic *Escherichia coli* isolated from broiler chickens. *Vet World* 2017; **10**: 1167–72. https://doi.org/10.14202/vetworld.2017.1167-1172

28 Collignon P, Aarestrup FM, Irwin R *et al*. Human deaths and third-generation cephalosporin use in poultry, Europe. *Emerg Infect Dis* 2013; **19**: 1339–40. https://doi.org/10.3201/eid.1908.120681

29 Shirakawa T, Sekizuka T, Kuroda M *et al*. Comparative genomic analysis of third-generation-cephalosporin-resistant *Escherichia coli* harbouring the blaCMY-2-positive IncI1 group, IncB/O/K/Z, and IncC plasmids isolated from healthy broilers in Japan. *Antimicrob Agents Chemother* 2020; **64**: e02385-19. https://doi.org/10.1128/AAC.02385-19

30 Webster P. The perils of poultry. *CMAJ* 2009; **181**: 21–4. https://doi.org/10.1503/cmaj.091009

31 Overdevest I, Willemsen I, Rijnsburger M *et al*. Extended-spectrum β-lactamase genes of *Escherichia coli* in chicken meat and humans, The Netherlands. *Emerg Infect Dis* 2011; **17**: 1216–22. https://doi.org/10.3201/eid1707.110209

32 Shah DH, Board MM, Crespo R *et al*. The occurrence of Salmonella, extended-spectrum β-lactamase producing *Escherichia coli* and carbapenem resistant non-fermenting Gram-negative bacteria in a backyard poultry flock environment. *Zoonoses Public Health* 2020; **67**: 742–53. https://doi.org/10.1111/zph.12756

33 Khoshbakht R, Seifi S, Raeisi M. Antibiotic susceptibility and high prevalence of extended spectrum beta-lactamase producing *Escherichia coli* in Iranian broilers. *Rev Med Vet (Toulouse)* 2016; **167**: 133–7. https://core.ac.uk/download/52205608.pdf

34 Lemlem M, Aklilu E, Mohammed M *et al*. Molecular detection and antimicrobial resistance profiles of Extended-Spectrum Beta-Lactamase (ESBL) producing *Escherichia coli* in broiler chicken farms in Malaysia. *PLoS One* 2023; **18**: e0285743. https://doi.org/10.1371/JOURNAL.PONE.0285743

35 Musicha P, Feasey NA, Cain AK *et al*. Genomic landscape of extended-spectrum β-lactamase resistance in *Escherichia coli* from an urban African setting. *J Antimicrob Chemother* 2017; **72**: 1602–9. https://doi.org/10.1093/JAC/DKX058

36 Choonara FE, Haldorsen BC, Janice J *et al*. Molecular epidemiological characterisation of ESBL- and plasmid-mediated AmpC-producing *Escherichia coli* and *Klebsiella pneumoniae* at Kamuzu Central Hospital, Lilongwe, Malawi. *Trop Med Infect Dis* 2022; **7**: 245. https://doi.org/10.3390/tropicalmed7090245

37 Larbi RO, Ofori LA, Sylverken AA *et al*. Antimicrobial resistance of *Escherichia coli* from broilers, pigs, and cattle in the Greater Kumasi Metropolis, Ghana. *Int J Microbiol* 2021; **2021**: 5158185. https://doi.org/10.1155/2021/5158185

38 Aworh MK, Kwaga J, Okolocha E *et al*. Extended-spectrum ß-lactamase-producing *Escherichia coli* among humans, chickens and poultry environments in Abuja, Nigeria. *One Health Outlook* 2020; **2**: 8. https://doi.org/10.1186/s42522-020-00014-7

39 Kiiti RW, Komba EV, Msoffe PL *et al*. Antimicrobial resistance profiles of *Escherichia coli* isolated from broiler and layer chickens in Arusha and Mwanza, Tanzania. *Int J Microbiol* 2021; **2021**: 6759046. https://doi.org/10.1155/2021/6759046

40 Stokes HW, Gillings MR. Gene flow, mobile genetic elements and the recruitment of antibiotic resistance genes into Gram-negative pathogens. *FEMS Microbiol Rev* 2011; **35**: 790–810. https://doi.org/10.1111/j.1574-6976.2011.00273.x

41 Moura de Sousa J, Lourenço M, Gordo I. Horizontal gene transfer among host-associated microbes. *Cell Host Microbe* 2023; **31**: 513–27. https://doi.org/10.1016/j.chom.2023.03.017