Open Veterinary Journal, (2025), Vol. 15(1): 171-178
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)

Original Article
DOI: *10.5455/OVJ.2025.v15.i1.16*

Submitted: 28/08/2024 Accepted: 02/12/2024 Published: 31/01/2025

Molecular identification of tetracycline resistance genes in *Escherichia coli* isolates from internal organs of swine sold on Abakaliki, Nigeria

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ABSTRACT

Background: Swine is one of the major sources of protein to humans worldwide; antimicrobial-resistant *Escherichia coli* has become a global public health challenge affecting both humans and livestock due to the presence of tetracycline resistance genes.

Aim: This study focused on molecular identification of tetracycline resistance genes (*tet* A and B) in *E. coli* isolates from internal organs of swine sold in a slaughterhouse at Abakaliki, Ebonyi State, Nigeria.

Methods: A total of 75 internal organs of swine samples were collected from slaughterhouses. Standard microbiological procedures were employed to evaluate the samples bacteriologically. Using the disk diffusion method, antibiotic susceptibility testing was conducted on *E. coli* against specific classes of antibiotics, and the multiple antibiotic resistance index was calculated. The polymerase chain reaction was utilized for the molecular identification of the tetracycline resistance genes, specifically *tet* A and B.

Results: Out of the 75 samples analyzed, 24 of 75 were positive for *E. coli* with an overall prevalence of 24/75 (32.0%). The small intestine and colon had higher percentages of *E. coli* isolates 6/15 (40.0%). However, *E. coli* isolates were resistant to erythromycin, tetracycline, and ampicillin which ranged from 20.0% to 75.0%, and susceptible to gentamycin and ciprofloxacin at a range of 75.0%–100.0%. Exactly, 8 (33.3%) isolates were both multidrug and tetracycline-resistant. The presence of *tet* A 6/8 (75.0%), *tet* B 5/8 (62.5%), and *tet* A and B 4/8 (50.0%) was reported.

Conclusion: Multidrug and tetracycline resistance genes have been observed in *E. coli* isolated from internal organs of swine and are of public health concern.

Keywords: Tetracycline resistance genes, *E. coli*, Internal organs, Swine, Public health.

Introduction

The lower intestine of both humans and animals frequently contains the rod-shaped, Gram-negative bacterium *Escherichia coli*. In Nigerian pig farms, *E. coli* illness causes significant productivity losses in both pre- and post-weaned piglets (Egbule *et al*., 2021). Resistance profiles of *E. coli* recovered from food animals, such as pigs and poultry, have a strong association with those of isolates from bloodstream infections in people (Vieira *et al*., 2011; Effendi *et al*., 2018). Piglets and pigs suffering from livestock illnesses such as *E. coli* disease are a serious danger to the profitability and long-term viability of the Nigerian pig industry. Nigerians consume a lot of pork, and pigs carry a higher risk of spreading multidrug-resistant *E. coli* to people through the food chain (Egbule *et al*., 2021). Highly adapted organisms called *E. coli* inhabit the gastrointestinal tracts of both people and animals,

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thriving in conditions such as water, dirt, sediment, and feces.

The presence of *E. coli* is a reliable indicator of environmental fecal contamination and is essential for tracking the spread of antimicrobial resistance (AMR) genes among bacterial communities (Tyasningsih *et al*., 2022; Mustika *et al*., 2024). In terms of the transmission of AMR, the relationships between humans, animals, and the environment are complex (Oloso *et al*., 2018; Yanestria *et al.*, 2022). Nearly similar IncI1 plasmids encoding third-generation cephalosporin resistance determinants are carried by the genetically different *E. coli* isolates from humans and animals. This plasmid aids in the transmission of multidrug-resistant organisms from food animals (such as chickens and pigs) to humans (de Been *et al*., 2014). Drug-resistant *E. coli* can occur via gene transfer in a variety of settings, as a result of contaminating livestock transport vehicles, trading venues, herd expansion through the addition of new animals, and slaughterhouse lairage (Schmithausen *et al*., 2018). Since surface water, wastewater, and drinking water can all be contaminated with *E. coli*, there is a chance that MDR will be transferred from the environment to pigs. It is known that holding pens in stables and lairage in abattoirs are important hubs for the spread of Enterobacteriaceae that produce ESBLs throughout the pig and other animal production chain (Schmithausen *et al*., 2015; Ugbo *et al*., 2023).

Food handlers are in danger of spreading resistant bacteria, and food processing settings are thought to be significant intermediate reservoirs and vectors of AMR bacteria (Oniciuc *et al*., 2019). Some data suggested the possible transfer of MDR *E. coli* clones between pigs and piggery workers (de Beenet *et al*., 2014). Multidrug resistance *E. coli* were considered as one of the major threats to animal and human health (Kendek *et al*., 2024). It also causes colibacillosis, a disease that commonly affects pigs from birth to weaning and is characterized by white to yellow diarrhea (Hartadi *et al*., 2020). Resistant to antibiotics humans can get *E. coli* from animals through the environment, direct contact, or the food chain (Schwaiger *et al*., 2012). The discovery of antibiotic resistance genes in *E. coli* extracted from animals has garnered significant interest, particularly in organisms capable of transmitting resistance genes (Effendi *et al*., 2021).

The use of antibiotics, such as bacitracin, lincomycin, neomycin/ oxytetracycline, and penicillin in promoting animal growth, has contributed to multidrug resistance worldwide. Antibiotic resistance in bacteria may arise as a result of the frequent and inappropriate use of these drugs (Gholami-Ahangaran *et al*., 2021). Gram-negative bacteria, *E. coli,* used an efflux pump system as one of the mechanisms of resistance to tetracycline, and tetracycline-resistant genes that can be found in *E. coli* include *tetA*, *tetB*, *tetC*, *tetD*, *tetE*, *tetG*; thus, *tetM*, *tetO*, and *tetS* encode ribosomal protection systems (Sigirci *et al*., 2020). *Tet* genes are responsible for the emergence of resistance to tetracycline antibiotics in *E. coli*. Studies have demonstrated the presence of the *tetA*, *tetB*, and *tetM* genes in the effluent of pig slaughterhouses in Germany and Portugal where *tetA*, *tetB*, *tetM*, and *tetK* genes were detected in the waste of a pig farm, and the *tetA*, *tetB*, *tetK*, *tetL*, *tetM*, *tetO*, and *tetA(P)* genes in the effluent of pig slaughterhouses (Pazra *et al.,* 2023). Additionally, information regarding genes responsible for tetracycline-resistant *E. coli* in swine is lacking in many parts of Nigeria including Abakaliki; thus, the need for this study on molecular identification of tetracycline resistance genes (*tet* A&B) in *E. coli* isolates from internal organs of swine sold in a slaughterhouse at Abakaliki, Ebonyi State, Nigeria.

Materials and Methods

Sample collection and processing

A total of 75 samples were collected from the swine slaughterhouses (15 each from the large intestine, small colon, kidney, liver, and small intestine of swine). The samples were collected from April 2023 to June 2023 and from both male and female pigs. Then, exactly 5 g of each sample was collected using a sterile knife and universal container, and all the samples were labeled with the unique sample number and date; the samples were transported to the laboratory at the Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki, for microbiological analysis. The collected samples were analyzed for the presence of *E. coli* by inoculating 5 g each of well-homogenized swine samples into different separate test tubes containing sterile buffered peptone water, and nutrient broth (Himedia) and incubated at 37°C for 24 hours. A loopful of the murky broth culture was aseptically seeded after being cultured overnight on sterile, solidified MacConkey agar (MAC) (Himedia) and Eosin methylene agar (EMB) (Merck USA). The samples were then incubated for 24 hours at 37°C. The distinctive appearance (color, consistency, and shape) of *E. coli* from positive cultures was used to identify them on the differential media. For Gram-staining response, hanging drop test (motility test), and biochemical profiling, the pink colonies on MAC and the greenish metallic shiny colonies on EMB were sub-cultured on sterilized solidified nutrient agar and incubated at 37°C for 24 hours to generate pure culture, following standard methods. The following biochemical tests were performed: the citrate utilization test (Simmon citrate agar), the oxidase test, and the indole test (Effendi *et al*., 2022).

Antibiotic susceptibility testing

The Kirby–Bauer disk diffusion method was used to determine the antibiotic susceptibility of the phenotypically determined *E. coli* isolates from the swine internal organs. The Mueller–Hinton agar medium (Merck USA) was made in compliance with

the guidelines provided by the manufacturer. A pure culture of *E. coli* isolates was cultured for overnight in nutrient broth, with the turbidity adjusted to 0.5 McFarland. After inoculating the plates, the bacterial isolates were left there for ten minutes for proper absorption on the plate. Antibiotics, which include fluoroquinolone (CIP: ciprofloxacin; 5 µg), macrolides (E: erythromycin; 30 µg), tetracycline (TE: tetracycline; 30 µg), beta-lactams (AMP: ampicillin; 30 µg), and aminoglycosides (CN: gentamycin; 500 µg), were placed on the surface of the Mueller–Hinton agar at a distance of 15 mm from the edge of the plate, 30 mm away from the center disk and incubated at 37°C for 18–24 hours. A calibrated transparent ruler was used to measure the inhibition zone diameters surrounding each antibiotic disk, and the results were reported in millimeters. The "resistant" and "sensitive" status of each bacterium was ascertained using a typical Clinical and Laboratory Standards Institute breakpoint table (CLSI, 2019).

Multiple antibiotic resistance index

Among the isolates resistant to three or more antimicrobial drugs, several antibiotic resistances were identified. The number of antibiotics to which an isolate is resistant (a) divided by the total number of antimicrobial agents the isolate was tested against (b) yields the MAR index for that isolate (Ejikeugwu *et al*., 2018).

Molecular identification of Tetracycline-resistant genes using PCR

PCR was used to further examine the tetracycline-resistant *E. coli* isolates for the presence of *tet* A and B genes. The pure culture of *E. coli* was inoculated in nutrient broth (TITAN Biotech, India) and cultured overnight at 37°C. The ZR fungal/bacterial DNA MiniPrep kit (manufactured by Zymo Research) was employed for the DNA extraction following the manufacturer's instructions. Industrial ready-to-use prepared PCR master mix solution (GoTaq Green Master Mix), which contains Taq DNA polymerase, dNTPs, MgCl2, and a reaction buffer at a concentration of 12.5 µl were used. The primers used were acquired from Whitehead Scientific Ltd, Cape Town, South Africa. A C1000 thermo cycler machine (Bio-Red) was used to conduct the PCR reactions for the *tet* A and B genes. The primers sequences used are *tet* A–F: GCT ACA TCC TGC TTG CCT TC; *tet* A–R: CAT AGA TCG CCG TGA AGA GG with 210 base pair; *tet* B–F: TTG GTT AGG GGC AAG TTT TG; *tet* B–R: GTA ATG GGC CAA TAA CAC CG with 659 base pair and PCR methods was according to previously described (Kallau*et al*., 2018; Gholami-Ahangaran*et al*., 2021). PCR products were visualized using 1.5% agarose gel electrophoresis and photographed under a UV transilluminator using a gel documentation system.

Ethical approval

Pork samples were acquired from the slaughterhouse; hence, ethical approval was not necessary. Samples were collected from the slaughterhouse, as per the standard collection procedure.

Results

Out of the 75 samples collected to determine the presence of *E. coli* in swine, 24/75 (32.0%) were positive. However, *E. coli* isolates identified had a different prevalence; the large intestine harbored 4/15 (26.7%), the small colon had 6/15 (40.0%), the kidney had 5/15 (33.3%), the liver harbored 3/15 (20.0%), and the small intestine had 6/15 (40.0%) (Table 1). Thus, *E. coli* isolates from the large intestine, kidney, liver, small colon, and small intestine had different resistance patterns to erythromycin, tetracycline, and ampicillin, which ranged from 20.0% to 75.0%. The *E. coli* isolates studied showed great susceptibility to gentamycin and ciprofloxacin. The susceptibility patterns observed among the isolates ranged from 75.0% to 100.0% (Table 2). In addition, three major multiple antibiotic resistance index/patterns were observed among the *E. coli* isolates, which are presented as TE-CN-E-AMP, TE-E-AMP-CIP, and TE-E-AMP with an average multiple antibiotic resistance index (MARI) of 0.70 (Table 3). Out of the 24 *E. coli* isolates studied, 8/24 (33.3%) were resistant to tetracycline antibiotics and were further studied for the presence of *tet* A and B genes. Exactly, 6/8 (75.0%) *E. coli* harbored *tet* A, 5/8 (62.5) had *tet* B, and 4/8 (50.0%) harbored both *tet* A and *tet* B genes (Table 4). The presence of *tet* A and *tet* B genes with the molecular size of 210 bp and 659 bp, respectively.

Discussion

Escherichia coli is a major bacterium that colonizes the intestine of piglets at birth and in disease conditions;

Table 1. Frequency of *E. coli* isolates from internal organs of swine sold in slaughterhouses at Abakaliki.

Sample source	No. of for sample	No. positive for *E. coli* (%)	Percentage prevalence (%)
Large intestine	15	4	26.7
Small colon	15	6	40.0
Kidney	15	5	33.3
Liver	15	3	20.0
Small intestine	15	6	40.0
Total	75	24	32.0

Table 2. Antimicrobial susceptibility profile of *E. coli* isolates from internal organs of swine sold in slaughterhouses at Abakaliki.

Source	TET		CN		E		AMP		CIP	
	S	R	S	R	S	R	S	R	S	R
Large intestine	2 (50.0)	2 (50.0)	3 (75.0)	1 (25.0)	2 (50.0)	2 (50.0)	1 (25.0)	3 (75.0)	4 (100)	0 (0.0)
Small colon	4 (66.7)	2 (33.3)	5 (83.3)	1 (16.7)	3 (50.0)	3 (50.0)	3 (50.0)	3 (50.0)	5 (100)	0 (0.0)
Kidney	4 (80.0)	1 (20.0)	5 (100)	0 (0.0)	3 (60.0)	2 (40.0)	2 (40.0)	3 (60.0)	4 (80.0)	1 (20)
Liver	2 (66.7)	1 (33.3)	3 (100)	0 (0.0)	2 (66.7)	1 (33.3)	1 (33.3)	2 (66.7)	3 (100)	0 (0.0)
Small intestine	4 (66.7)	2 (33.3)	5 (83.3)	1 (16.7)	4 (66.7)	2 (33.3)	3 (50.0)	3 (50.0)	5 (83.3)	1 (16.7)

CIP = ciprofloxacin; E = erythromycin; TE = tetracycline; AMP = ampicillins; and CN = gentamycin.

Table 3. Multiple antibiotic resistance patterns of *E. coli* isolates from internal organs of swine sold in slaughterhouses at Abakaliki.

Isolates code	Isolates	Number of antibiotics that isolates were resistant to (a)	Total number of antibiotics tested (b)	MAR index (a/b)
LI 3	*E. coli*	TE-E-AMP	5	0.60
LI 9	*E. coli*	TE-CN-E-AMP	5	0.80
SC 1	*E. coli*	TE-CN-E-AMP	5	0.80
SC 8	*E. coli*	TE-E-AMP	5	0.60
K 2	*E. coli*	TE-E-AMP-CIP	5	0.80
L 4	*E. coli*	TE-E-AMP	5	0.60
SI 3	*E. coli*	TE-CN-E-AMP	5	0.60
SI 14	*E. coli*	TE-E-AMP-CIP	5	0.80
Average index				0.70

LI = large intestine; SC = small colon; K = kidney; L = liver; SI = small intestine; CIP = ciprofloxacin; E = erythromycin; TE = tetracycline; AMP = ampicillins; and CN = gentamycin.

Table 4. Occurrence of tetracycline resistance genes in *E. coli* from internal organs of swine.

Sample source	No *E. coli* isolates	No tetracycline resistant *E. coli* isolates studied in PCR (%)	*tet* A gene (%)	*tet* B gene (%)	*tet* A abd B gene (%)
Large intestine	4	2 (50.0)	2 (100.0)	2 (100.0)	2 (100.0)
Small colon	6	2 (33.3)	2 (100.0)	1 (50.0)	1 (50.0)
Kidney	5	1 (20.0)	0 (0.0)	1 (100.0)	0 (0.0)
Liver	3	1 (33.3)	1 (100.0)	1 (100.0)	1 (100.0)
Small intestine	6	2 (33.3)	1 (50.0)	0 (0.0)	0 (0.0)
Total	24	8 (33.3)	6 (75.0)	5 (62.5)	4 (50.0)

it creates serious problems for the pig farm managers/owners. This research shows that the prevalence of *E. coli* from swine slaughtered at Abakaliki is 24 (32.0%) out of the 75 swine samples analyzed. A study on *E. coli* causing diseases in pig farms in Bulgarian has reported 52 (30.4%) of *E. coli* from fecal samples of swine (Dimitrova *et al*., 2021) and is by the findings of this study. According to the study, 50.0% of *E. coli* cases in Southern Nigeria were from retail stores and pig slaughterhouses (Egbule *et al*., 2021). The presence of *E. coli* isolates has been reported from livestock, which includes pigs and chickens (Wibisono *et al*., 2020; Yanestria *et al*., 2022) and pathogenic *E. coli* from healthy pigs in Malang district, East Java, Indonesia (Effendi *et al*., 2022) and pig farms at Bulgarian (Dimitrova *et al*., 2021). It was confirmed by earlier research that pathogenic *E. coli* isolates can travel from the natural environment to humans or other livestock

if they are excreted by animals (Dohmen *et al*., 2017). Studies have shown that *E. coli* can contaminate pigs, regardless of whether meat samples are obtained at the slaughterhouse or after processing (Bassitta *et al*., 2022).

However, *E. coli* isolates from the large intestine, kidney, liver, small colon, and small intestine were 20.0%–75.0% resistant to erythromycin, tetracycline, and ampicillin and 75.0%–100.0% susceptible to gentamycin and ciprofloxacin. This study also identified the presence of *E. coli* isolates that were resistant to β-lactam (ampicillin) antibiotics and agrees with the observation that *E. coli* in food-producing animals conferring resistance to β-lactam antimicrobials (Reich *et al*., 2013). The reason for this resistance possessed by this organism could be that some of these antimicrobial agents used as growth promoters and treatment of food animals in veterinary medicine range from oxytetracycline, penicillin, and ampicillin have been abused by the farms (Widodo *et al*., 2020). *E. coli* that was resistant to tetracycline antibiotics was also discovered in this study and is in line with the observation that *E. coli* isolates from livestock can resist most antibiotics such as aminoglycosides, tetracyclines, chloramphenicol, trimethoprim, sulphonamides, and quinolones (Wibisono *et al*., 2021). The *E. coli* isolates from this study had antibiotic antibiotic-resistant ranging from 20.0% to 75.0%, and this is akin to the report that *E. coli* isolates from pigs had a resistant range of 53.0%–75.0% (Dimitrova *et al*., 2021).

A study done in Uganda on *E. coli* isolates from pig slaughterhouses presented antibiotic resistance to amoxicillin (30.4%), erythromycin (34.8%), and ciprofloxacin (100%) susceptibility (Katushabe *et al*., 2022) and it is by the observation of this research. Another researcher discovered that antimicrobial resistance was dominated by tetracycline (69.1 %) (Ugbo *et al*., 2023). Regardless of a strain's virulence characteristics, antimicrobial resistance may pose health problems since carriers may act as a conduit for genetic transfer within the gastrointestinal tract. Research has demonstrated that resistant bacteria, such as *E. coli*, can survive in the gastrointestinal tract after being consumed orally through pork (Enne *et al*., 2008). Managers of swine farms frequently deal with the issue of bacterial pathogens causing disease in pigs. Antibiotics are now used to prevent and treat diseases as a result of this (Van *et al*., 2020).

This study has reported the presence of multidrug-resistant *E. coli* and the isolates presented MDR as follows TE-CN-E-AMP, TE-E-AMP-CIP, and TE-E-AMP with average MARI as 0.70. A retrospective study revealed that 43 *E. coli* isolates had an 88.4% multidrug resistance rate (Kakooza *et al*., 2021). There have been reports of multidrug-resistant bacteria in various livestock, particularly in pigs and dairy cattle (Byaruhanga *et al*., 2022). The occurrence of multidrug resistance in livestock might be increased by the irrational and inappropriate use of antimicrobial drugs (Larb *et al*., 2021). Antibiotics are still used as growth boosters on farm animals such as pigs, cattle, poultry, and birds in certain countries that have not outlawed their usage. This has significantly accelerated the spread of antibiotic resistance in livestock (Yang *et al*., 2019). Antibiotic-resistant bacteria have the detrimental effect of making therapy for bacterial illnesses more difficult or perhaps failing. Ineffective therapy affects the duration of treatment, the usage of more costly medications, and, of course, the expenses incurred (Widodo *et al*., 2020).

Exactly, 8 (33.3%) *E. coli* isolates were multidrug-resistant and tetracycline-resistant and were further studied for the presence of *tet* A and *tet*B genes. However, the *E. coli* was reported to harbor *tet* A gene 6/8 (75.0%); *tet* B gene 5/8 (62.5%); and both *tet* A and B genes 4/8 (50.0%). A high prevalence of the *tet* A gene was observed when compared with *tet* B gene and agrees with the observation of Alam *et al*. (2023) who reported that 84.0% of the tetracycline-resistant *E. coli* isolates encode *tet* A gene. Different types of tetracycline genes, which include *tet*(A), *tet*(B), *tet*(C), *tet*(D), and *tet*(E), have been previously reported by several studies to cause tetracycline resistance in bacteria such as *E. coli* (Sigirci *et al*., 2020; Pazra *et al*., 2023). Another study carried out on *E. coli* isolated from a pig farm in Indonesia reported the presence of multidrug and tetracycline resistance *E. coli* to harbor *tet* A (23.0%), *tet* E (46.0), and *tet* A and B (23.0%) genes (Kallau *et al*. 2018). The *tet*A gene was more prevalent (17/18) at 94%, followed by the *tet*B gene at (10/18) 56% in *E. coli* (Indrawati *et al*., 2021), and is by the detection of *tet* A gene 6/8 (75.0%) and *tet* B gene 5/8 (62.5%) in this research. A study done in clinical and non-clinical tetracycline resistance *E. coli* isolates in Nigeria detected *tet*A (34.8%), *tet* B (2.2%), and *tet* A and B (14.8%) (Perewari *et al*., 2022). The *E. coli* isolates that were resistant to tetracycline and harboring *tet*A (42.3%), *tet*B (46.2%), and *tet*A and B (11.5%) genes were reported in healthy and diarrheic pet birds (Gholami-Ahangaran *et al*., 2021). Thus, this research has found that tetracycline resistance *E. coli* in swine is due to sales, use of tetracycline antibiotics, and cross-resistant antimicrobials in Abakaliki metropolis. This study also shows that there is a correlation between the use of tetracycline and resistance of tetracycline.

Conclusion

In conclusion, this study has reported the presence of antimicrobial-resistant *E. coli* in swine. Multidrug-resistant and tetracycline resistance genes (*tet* A, *tet* B, both *tet* A and B genes) were also reported in *E. coli* isolates from internal organs of swine for the first time in Abakaliki and is of public health concern. Thus, swine should be considered a potential source of MDR organisms capable of causing public health threats/hazards. This discovery has impacted optimizing the

use of antibiotics as growth promoters, and disease treatment and the data obtained are also helpful in reducing the increasing incidence and spread of antimicrobial resistance among food animals, humans, and their environment at the grass root level and even the remote and rural regions of Nigeria that deal with the practice of pig/swine farm and other livestock. We therefore recommend strong stringent antibiotic administering policies for swine and surveillance on the emergence of antimicrobial resistance in livestock.

Acknowledgments

The authors thank the Universitas Airlangga.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding

The authors would like to acknowledge Ebonyi State University, Abakaliki Nigeria, and Lembaga Penelitian dan Pengabdian Masyarakat Universitas Airlangga, Indonesia, for their support. This study was partly supported by the International Research Consortium, Lembaga Penelitian dan Pengabdian Masyarakat, Universitas Airlangga, Surabaya, Indonesia Year 2024 with grant number: 171/UN3.LPPM/PT.01.03/2024.

Author's contributions

AIU and ENU: Conceived, designed, and coordinated the study. IBM and RCO: Designed data collection tools, supervised the field sample and data collection, and laboratory work as well as data entry. WT, HMR, and MHE: Validation, supervision, and formal analysis. BNA and SMY: Contributed reagents, materials, and analysis tools. KHPR, FE, and ARK: Carried out the statistical analysis and interpretation and participated in the preparation of the manuscript. All authors have read, reviewed, and approved the final manuscript.

Data availability

All data are available in the manuscript.

References

Alam, G.S., Hassan, M.M., Ahaduzzaman, M., Nath, C., Dutta, P., Khanom, H., Khan, S.A., Pasha, M.R., Islam, A. and Magalhaes, R.S. 2023. Molecular detection of tetracycline-resistant genes in multi-drug-resistant *Escherichia coli* isolated from broiler meat in Bangladesh. Antibiotics 12(2), 418.

Bassitta, R., Kronfeld, H., Bauer, J., Schwaiger, K. and Hölzel, C. 2022. Tracking antimicrobial resistant *E. coli* from pigs on farm to pork at slaughter. Microorganisms 10(8), 1485.

Byaruhanga, J., Ssebunya, Y., Vudriko, P. and Rwego, I.B. 2022. Antimicrobial resistance patterns and bovine sub-clinical mastitis burden in low and high tick acaricide resistance regions of Uganda. Open J. Vet. Med. 12(8), 71–87.

Clinical and Laboratory Standards Institute. 2018. M100 performance standards for antimicrobial susceptibility testing. 28th ed. Clinical and Laboratory Standards Institute. www.clsi.org. Published in Wayne City, Pennsylvania (PA).

De Been, M., Lanza, V.F., de Toro, M., Scharringa, J., Dohmen, W., Du, Y., Hu, J., Lei, Y., Li, N. and Tooming-Klunderud, A. 2014. Dissemination of cephalosporin resistance genes between *Escherichia coli* strains from farm animals and humans by specific plasmid lineages. PLoS Genet. 10(12), 90–95.

Dimitrova, L., Kaleva, M., Zaharieva, M.M., Stoykova, C., Tsvetkova, I., Angelovska, M., Ilieva, Y., Kussovski, V., Naydenska, S. and Najdenski, H. 2021. Prevalence of antibiotic-resistant *Escherichia coli* isolated from swine faeces and lagoons in Bulgaria. Antibiotics 10(8), 940–946.

Dohmen, W., Dorado-García, A., Bonten, M.J.M., Wagenaar, J.A., Mevius, D. and Heederik, D.J. 2017. Risk factors for ESBL-producing *Escherichia coli*on pig farms: a longitudinal study in the context of reduced use of antimicrobials. PLoS One 12(3), 174–184.

Effendi**,** M.H., Harijani, N., Yanestria, S.M. and Hastutiek, P. 2018. Identification of Shiga toxin-producing *Escherichia coli* in raw milk samples from dairy cows in Surabaya, Indonesia. Philipp. J. Vet. Med. 55(SI), 109–114.

Effendi, M.H., Hartadi, E.B., Witaningrum, A.M., Permatasari, D.A. and Ugbo, E.N. 2022. Molecular identification of *bla*TEM gene of extend¬ed-spectrum beta-lactamase-producing *Escherichia coli* from healthy pigs in Malang district, East Java, Indonesia. J. Adv. Vet. Anim. Res. 9(3), 447–452.

Effendi, M.H., Tyasningsih, W., Yurianti, Y.A., Rahmahani, J., Harijani, N. and Plumeriastuti, H. 2021. Presence of multidrug resistance (MDR) and extended-spectrum beta-lactamase (ESBL) of *Escherichia coli* isolated from cloacal swab of broilers in several wet markets in Surabaya, Indonesia. Biodiversitas 22(1), 304–309.

Egbule, O.S., Iweriebor, B.C. and Odum, E.I. 2021. Beta-lactamase-producing *Escherichia coli* isolates recovered from pig handlers in retail shops and abattoirs in selected localities in Southern Nigeria: implications for Public Health. Antibiotics 10(1), 9.

Ejikeugwu, C., Esimone, C., Iroha, I.R. and Adikwu, M. 2018. First detection of FOX-1 AmpC β-lactamase gene expression among *Escherichia coli* isolated from abattoir samples in Abakaliki, Nigeria. Oman Med. J. 33(3), 243–249.

Enne, V.I., Cassar, C., Sprigings, K., Woodward, M.J. and Bennett, P.M. 2008. A high prevalence of antimicrobial resistant *Escherichia coli* isolated from pigs and a low prevalence of antimicrobial resistant *E. coli* from cattle and sheep in Great Britain at slaughter. FEMS Microbol. Lett. 278(2), 193–199.

Gholami-Ahangaran, M., Karimi-Dehkordi, M., Miranzadeh-Mahabadi, E. and Ahmadi-Dastgerdi,

A. 2021. The frequency of tetracycline resistance genes in *Escherichia coli* strains isolated from healthy and diarrheic pet birds. Iran J. Vet. Res. 22(4), 337–341.

Hartadi, E.B., Effendi, M.H., Plumeriastuti, H., Sofiana, E.D., Wibisono, F.M. and Hidayatullah, A.R. 2020. A review of enterotoxigenic *Escherichia coli* infection in piglets: public health importance. Syst. Rev. Pharm. 11(9), 687–698.

Indrawati, A., Khoirani, K., Setiyaningsih, S., Affif, U., Safika, S. and Ningrum, G. 2021. Detection of tetracycline resistance genes among *Escherichia coli* isolated from layer and broiler breeders in West Java, Indonesia. Trop. Anim. Sci. J. 44(3), 267–272.

Kallau, N.H.G., Wibawan, I.W.T., Lukman, D.W. and Sudarwanto, M.B. 2018. Detection of multi-drug resistant (MDR) *Escherichia coli* and tet gene prevalence at a pig farm in Kupang, Indonesia. J. Adv. Vet. Anim. Res. 5(4), 388–396.

Kakooza, S., Muwonge, A., Nabatta, E., Eneku, W., Ndoboli, D. and Wampande, E. 2021. A Retrospective analysis of antimicrobial resistance in pathogenic *Escherichia coli* and *Salmonella* spp. isolates from poultry in Uganda. Int. J. Vet. Sci. Med. 9(1), 11–21.

Katushabe, P., Byamukama, B. and Byaruhanga, J. 2022. Burden of multidrug-resistant *Escherichia coli* in pigs slaughtered in Uganda and its implication on veterinary public health. Open. J. Vet. Med. 2022(12), 187–200.

Kendek, I.A., Putri, M.F.R., Wibisono, F.J., Effendi, M.H., Tyasningsih, W., Ugbo, E.N. and Agumah, N.B. 2024. Molecular detection of *hly*F gene on multidrug resistance of avian pathogenic *Escherichia coli* isolated from ducks on wet markets of Surabaya, Indonesia. Biodiversitas 25(3), 1246–1252.

Larbi, R.O., Ofori, L.A., Sylverken, A.A., Ayim-Akonor, M. and Obiri-Danso, K. 2021. Antimicrobial resistance of *Escherichia coli* from broilers, pigs, and cattle in the greater Kumasi Metropolis, Ghana. Int. J.Microbiol. 7(2), 31–39.

Mustika, Y.R., Kinasih, K.N., Effendi, M.H., Puspitasari, Y., Kurniawan, S.C., Khairullah, A.R., Samodra, M.E.E., Hasib, A., Agustin, A.L.D., Moses, I.B. and Silaen, O.S.H. 2024. Molecular detection of extended-spectrum β-lactamase-producing *Escherichia coli* from bat caves on Lombok Island. Open Vet. J. 14(2), 699–706.

Oloso, N.O., Fagbo, S., Garbati, M., Olonitola, S.O., Awosanya, E.J., Aworh, M.K., Adamu, H., Odetokun, I.A. and Fasina, F.O. 2018. Antimicrobial resistance in food animals and the environment in Nigeria: a review. Int. J. Environ. Res. Public Health 15(6), 1284.

Oniciuc, E.A., Likotrafiti, E., Alvarez-Molina, A., Prieto, M., López, M. and Alvarez-Ordóñez, A. 2019. Food processing as a risk factor for antimicrobial resistance spread along the food chain. Curr.Opin. Food. Sci. 30(12), 21–26.

Pazra, D.F., Latif, H., Basri, C., Wibawan, I.W.T. and Rahayu, P. 2023. Distribution analysis of tetracycline resistance genes in *Escherichia coli* isolated from floor surface and effluent of pig slaughterhouses in Banten Province, Indonesia, Vet. World 16(3), 509–517.

Perewari, D.O., Otokunefor, K. and Agbagwa, O.E. 2022. Tetracycline-resistant genes in *Escherichia coli* from clinical and nonclinical sources in Rivers State, Nigeria. Int. J. Microbiol. 2022(7), ID9192424.

Reich, F., Atanassova, V. and Klein, G. 2013. Extended spectrum beta-lactamase and AmpC-producing Enterobacteria in healthy broiler chickens, Germany. Emerg. Infect. Dis. 19(8), 1253–1259.

Schmithausen, R.M., Schulze-Geisthoevel, S.V., Heinemann, C., Bierbaum, G., Exner, M., Petersen, B. and Steinhoff-Wagner, J. 2018. Reservoirs and transmission pathways of resistant indicator bacteria in the Biotope pig stable and along the food chain: a review from a One Health Perspect. Sustain. 10(11), 3967–3969.

Schmithausen, R.M., Schulze-Geisthoevel, S.V., Stemmer, F., El-Jade, M., Reif, M., Hack, S., Meilaender, A., Montabauer, G., Fimmers, R. and Parcina, M. 2015. Analysis of transmission of MRSA and ESBL-E among pigs and farm personnel. PLoS One 10(9), 76–81.

Sigirci, B.D., Celik, B., Halac, B., Adiguzel, M.C., Kekec, I., Metiner, K., Ikiz, S., Bagcigi, A.F., Ozgur, N.Y., Ak, S. and Kahraman, B.B. 2020. Antimicrobial resistance profiles of *Escherichia coli* isolated from companion birds. J. King Saud Univ. Sci. 32(1), 1069–1073.

Tyasningsih, W., Ramandinianto, S.C., Ansharieta, R., Witaningrum, A.M., Permatasari, D.A., Wardhana, D.K., Effendi, M.H. and Ugbo, E.N. 2022. Prevalence and antibiotic resistance of *Staphylococcus aureus* and *Escherichia coli* isolated from raw milk in East Java, Indonesia. Vet. World 15(8), 2021–2028.

Ugbo, E.N., Jacob, J.I., Effendi, M.H., Witaningrum, A.M., Agumah, B.N., Ugbo, A.I. and Moses, B.I. 2023. Poultry slaughterhouse wastewater as reservoirs for spreading extended-spectrum beta-lactamase-producing *Escherichia coli*in Abakaliki, Nigeria. Biodiversitas 24(9), 4960–4966.

Van, T.T.H., Yidana, Z., Smooker, P.M. and Coloe, P.J. 2020. Antibiotic use in food animals worldwide, with a focus on Africa: pluses and minuses. J. Glob. Antimicrob. Resist. 5(20), 170–173.

Vieira, A.R., Collignon, P., Aarestrup, F.M., McEwen, S.A., Hendriksen, R.S., Hald, T. and Wegener, H.C. 2011. Association between antimicrobial resistance in *Escherichia coli* isolates from food animals and blood stream isolates from humans in Europe: an

ecological study. Foodborne Pathog. Dis. 8(12), 1295–1301.

Wibisono, F.J., Sumiarto, B., Untari, T., Effendi, M.H., Permatasari, D.A. and Witaningrum, A.M. 2020. Pattern of antibiotic resistance on extended-spectrum beta-lactamases genes producing *Escherichia coli* on laying hens in Blitar, Indonesia. Biodiversitas 21(10), 4631–4635.

Wibisono, F.J., Sumiarto, B., Untari, T., Effendi, M.H., Permatasari, D.A. and Witaningrum, A.M. 2021. Molecular identification of *ctx* gene of extended spectrum betalactamases (ESBL) producing *Escherichia coli* on layer chicken in Blitar, indonesia. J. Anim. Plant. Sci. 31(4), 954–959.

Widodo, A., Effendi, M.H. and Khairullah, A.R. 2020. Extended-spectrum beta-lactamase (ESBL)-producing *Eschericia coli* from livestock. Syst. Rev. Pharm. 11(7), 382–392.

Yanestria, S.M., Dameanti, F.N.A.E.P., Musayannah, B.G., Pratama, J.W.A., Witaningrum, A.M., Effendi, M.H. and Ugbo, E.N. 2022. Antibiotic resistance pattern of extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* isolated from broiler farm environment in Pasuruan district, Indonesia. Biodiversitas 23(9), 4460–4465.

Yang, H., Paruch, L., Chen, X., van Eerde, A., Skomedal, H., Wang, Y., Liu, D. and Clarke, J.L. 2019. Antibiotic application and resistance in swine production in China: current situation and future perspectives. Front. Vet. Sci. 6(5), 136–137.