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Molecular detection of tetracycline resistance genes in an emerging *Enterobacteriaceae* isolate from earthen catfish ponds in Akure, Nigeria

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Abstract:

Background: Aquaculture environments are increasingly recognized as reservoirs for antimicrobial-resistant bacteria due to the routine use of antibiotics such as tetracyclines. Members of the family *Enterobacteriaceae* readily acquire and disseminate resistance determinants, posing risks to food safety and public health. In Nigeria, molecular data on tetracycline resistance in aquaculture systems remain limited. This study investigated the occurrence and molecular basis of tetracycline resistance among *Enterobacteriaceae* isolated from earthen catfish ponds in Akure, Nigeria.
Methodology: Composite samples were collected from five different locations within each of two randomly selected commercial-scale earthen catfish ponds and pooled for analysis. The resulting samples were cultured on selective media. Antibiotic susceptibility of four representative isolates was performed by the Kirby–Bauer disc diffusion method against ampicillin, cefuroxime, ciprofloxacin and tetracycline. The isolate exhibiting phenotypic tetracycline resistance was further characterized by 16S rRNA gene sequencing and phylogenetic analysis. Polymerase chain reaction (PCR) assay was used to detect tetracycline resistance genes (*tetA* and *tetM*) in the isolate.
Results: All four representative isolates were fully resistant to ampicillin and cefuroxime but susceptible to ciprofloxacin. One isolate (EP9) displayed complete resistance to tetracycline. Molecular identification revealed EP9 as *Escherichia albertii*, confirmed by phylogenetic clustering within a distinct *E. albertii* clade. PCR analysis detected the presence of the *tetA* gene (210 bp) in EP9, while *tetM* was not detected.
Conclusion: This study provides molecular evidence that earthen catfish ponds in Akure harbour tetracycline-resistant *Escherichia albertii*. The detection of the plasmid-mediated *tetA* gene underscores the role of aquaculture systems as reservoirs and dissemination hubs for clinically relevant antimicrobial resistance genes, highlighting the need for strengthened antibiotic stewardship and routine AMR surveillance in aquaculture.

Keywords: *Escherichia albertii,* Enterobacteriaceae, Aquaculture tetracycline resistance, Nigeria

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Détection moléculaire des gènes de résistance à la tétracycline chez un isolat émergent d'*Enterobacteriaceae* provenant d'étangs piscicoles de poissons-chats à fond de terre à Akure, Nigeria

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Résumé:

Contexte: Les environnements aquacoles sont de plus en plus reconnus comme des réservoirs de bactéries résistantes aux antimicrobiens en raison de l'utilisation courante d'antibiotiques tels que les tétracyclines. Les membres de la famille des *Enterobacteriaceae* acquièrent et disséminent facilement des déterminants de résistance, ce qui pose des risques pour la sécurité alimentaire et la santé publique. Au Nigeria, les données moléculaires sur la résistance aux tétracyclines dans les systèmes aquacoles restent limitées. Cette étude a examiné la présence et la base moléculaire de la résistance à la tétracycline chez des *Enterobacteriaceae* isolées d'étangs piscicoles de poissons-chats à fond de terre à Akure, au Nigeria.
Méthodologie: Des échantillons composites ont été prélevés à partir de cinq emplacements différents dans chacun de deux étangs piscicoles commerciaux à fond de terre sélectionnés au hasard et regroupés pour analyse. Les échantillons obtenus ont été cultivés sur des milieux sélectifs. La sensibilité aux antibiotiques de quatre isolats représentatifs a été déterminée par la méthode de diffusion sur disque de Kirby–Bauer contre l'ampicilline, le céfuroxime, la ciprofloxacine et la tétracycline. L'isolat présentant une résistance phénotypique à la tétracycline a ensuite été caractérisé par le séquençage du gène de l'ARNr 16S et une analyse phylogénétique. Une réaction en chaîne par polymérase (PCR) a été utilisée pour détecter les gènes de résistance à la tétracycline (*tetA* et *tetM*).
Résultats : Les quatre isolats représentatifs étaient entièrement résistants à l'ampicilline et au céfuroxime, mais sensibles à la ciprofloxacine. Un isolat (EP9) a montré une résistance complète à la tétracycline. L'identification moléculaire a révélé que l'isolat EP9 correspondait à *Escherichia albertii*, confirmé par son regroupement phylogénétique au sein d'un clade distinct de *E. albertii*. L'analyse PCR a détecté la présence du gène *tetA* (210 pb) chez EP9, tandis que le gène *tetM* n'a pas été détecté.
Conclusion: Cette étude fournit des preuves moléculaires indiquant que les étangs piscicoles à fond de terre d'Akure abritent *Escherichia albertii* résistante à la tétracycline. La détection du gène *tetA* médié par plasmide souligne le rôle des systèmes aquacoles comme réservoirs et centres de dissémination de gènes de résistance aux antimicrobiens cliniquement pertinents, mettant en évidence la nécessité de renforcer la gestion des antibiotiques et la surveillance régulière de la RAM dans l'aquaculture.

Mots-clés: *Escherichia albertii*, *Enterobacteriaceae*, aquaculture, résistance à la tétracycline, Nigeria.

Introduction:

Antimicrobial resistance (AMR) is one of the most urgent global public health threats of the 21st century, undermining the effectiveness of antibiotics and compromising the treatment of infectious diseases in both humans and animals. In 2019 alone, bacterial AMR was estimated to have caused approximately 1.27 million deaths worldwide (1), with projections suggesting that annual mortality could rise to 10 million by 2050 if current trends persist. Among bacterial groups of concern, members of the family *Enterobacteriaceae* are notable for their capacity to acquire and disseminate resistance determinants, often through mobile genetic elements such as plasmids and integrons.

The global escalation of multidrug-resistant *Enterobacteriaceae* has been reported in diverse environments, including clinical settings (2), agricultural areas (3), and aquatic ecosystems (4), where antibiotic use and environmental exposure contribute to resistance selection and propagation. Aquaculture, particularly in low- and middle-income countries, often employs antibiotics such as oxytetracycline for disease control and growth promotion. This practice exerts strong selective pressure on indigenous bacterial communities, thereby enhancing the persistence of antibiotic resistance genes (ARGs) in pond water, sediments, and cultured fish.

Tetracyclines are widely used in aquaculture due to their broad-spectrum activity and low cost; however, extensive application has been strongly linked to the emergence of tetracycline resistance and the widespread occurrence of *tet* genes in aquaculture environments, as evidenced by molecular studies using PCR-based detection methods (5).

Studies in fish farms and aquaculture environments have reported the occurrence of multiple *tet* genes among diverse bacterial taxa, including *Aeromonas*, *Pseudomonas* and *Enterobacteriaceae*, highlighting aquaculture systems as reservoirs and hotspots for tetracycline resistance determinants (6,7). Furthermore, the persistence of tetracycline resistance genes in pond environments appears to be influenced not only by ongoing antibiotic use but also by the intrinsic stability of resistance gene pools and horizontal gene transfer mechanisms (8). In Nigeria, investigations into antibiotic resistance among bacteria isolated from aquaculture environments remain limited, despite documented resistance to multiple antibiotics in related settings such as water distribution systems and animal production environments (9).

Given the economic and nutritional importance of catfish farming in southwestern Nigeria, understanding the molecular epidemiology of tetracycline resistance in *Enterobacteriaceae* from earthen ponds is essential for informing antibiotic stewardship, food safety, and public health risk assessments. This study, therefore, aimed to detect and characterize tetracycline resistance genes among *Enterobacteriaceae*

isolates from earthen catfish ponds in Akure, Nigeria, using PCR methodologies.

Materials and method:

Study area and sample collection:

Two commercial-scale earthen ponds used for catfish culture in Akure, Ondo State, Nigeria, were randomly selected for this study in May 2025. Water samples were collected from five different points within each pond to obtain representative coverage of the pond environment and minimize sampling bias caused by spatial variation in microbial distribution. Equal volumes of water from the five locations in each pond were pooled to form a composite sample and collected aseptically into sterile sampling bottles. The composite sampling approach was adopted to provide an overall representation of the microbial quality of each pond rather than conditions at a single point.

Immediately after collection, the samples were placed in an insulated container containing ice packs to maintain low temperature and limit changes in microbial composition during transport. The samples were transported promptly to the laboratory and processed within two hours of collection to ensure the reliability and integrity of the microbiological analysis.

Culture isolation and biochemical identification of *Enterobacteriaceae*:

Water samples were serially diluted up to 10^{-8} using sterile physiological saline, and 100 µl of each dilution was inoculated onto Chromogenic Coliform Agar (Oxoid, UK). Plates were incubated at 35°C and examined after 24 and 48 hours. The medium enables the presumptive differentiation of members of the *Enterobacteriaceae* based on chromogenic substrates that detect β-galactosidase and β-glucuronidase enzyme activities. After incubation, colonies were examined for characteristic colour reactions and morphological features. According to the manufacturer's colour chart for Chromogenic Coliform Agar, *Escherichia coli* colonies appeared dark blue to violet, while other coliform bacteria produced pink to red or Salmon red colonies. Non-coliform members of the *Enterobacteriaceae* appeared as colourless or pale colonies. Colony characteristics including size, shape, elevation, margin and surface appearance were also noted. From each plate, four colonies with distinct colour and morphological characteristics were randomly selected and purified by repeated streaking on nutrient agar to obtain pure cultures.

The purified isolates were Gram stained to confirm that the isolates were Gram-negative rods, consistent with members of the *Enterobacteriaceae* family. Further phenotypic identification was carried out using standard biochemical tests, including oxidase, catalase, indole production, methyl red (MR), Voges–Proskauer (VP), citrate utilization, urease, and motility tests as well as Triple Sugar Iron (TSI) agar test for carbohydrate fermentation and hydrogen sulphide production.

Antimicrobial susceptibility testing of isolates:

The antibiotic susceptibility of the purified isolates was performed by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar (Oxoid, UK) in triplicate, following CLSI guidelines. The antibiotics tested included tetracycline (30µg), cefuroxime (30µg), ciprofloxacin (5µg), and ampicillin (10µg). After incubation at 37°C for 24 hours, inhibition zone diameters were measured, and each isolate was categorized as susceptible, intermediate, or resistant based on CLSI interpretative criteria supplied with the antibiotic discs (Himedia). The isolate that was fully resistant to tetracycline was selected for further study.

16S rRNA gene sequencing and phylogenetic analysis:

Molecular identification of the tetracycline-resistant isolate was carried out through 16S rRNA gene sequencing. Genomic DNA was extracted and used as the template for PCR amplification. The PCR reaction was prepared using the Solis Biodyne 5X HOT FIREPol® Blend Master Mix. Each 25 µl reaction contained 1X Blend Master Mix buffer, 1.5 mM $MgCl_2$, 200 µM of each dNTP (Solis Biodyne), 25 pmol of each primer (BIOMERS, Germany), 2 units of HOT FIREPol® DNA polymerase with proofreading activity, 5 µl of template DNA, and sterile distilled water to volume. Amplification was performed in a Techne (3Prime series) thermal cycler with an initial denaturation at 95°C for 15 min, followed by 35 cycles of 95°C for 30 s, 61°C for 1 min, and 72°C for 1 min 30 s, and a final extension at 72°C for 10 min. The resulting amplicons were sequenced using the Nimagen BrilliantDye™ Terminator v3.1 Cycle Sequencing Kit (BRD3-100/1000) following the manufacturer's instructions. Sequence chromatograms were examined and edited using FinchTV software.

Taxonomic identification of the isolate was carried out by comparative analysis of its 16S rRNA gene sequence using the Basic Local Alignment Search Tool (BLAST) against the National Center for Biotechnology Information (NCBI) GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic analysis was performed using homologous sequences

retrieved from the NCBI Gene/Nucleotide database and inferred in MEGA version 12.1 using the Maximum Likelihood method. The tree was constructed using 1,000 bootstrap replicates, with bootstrap values indicating the robustness of the inferred relationships.

Detection of *tetA* and *tetM:*

PCR assays targeted tetracycline resistance genes using specific primers (Table 1). Conditions included an initial denaturation at 95°C for 5 min, 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 45 s, and a final extension at 72°C for 7 min. Products were analyzed on 1.5% agarose gels stained with ethidium bromide.

Results:

Frequency of *Enterobacteriaceae* isolated from earthen pond:

A total of two commercial-scale earthen catfish ponds were sampled in this study. From each pond, water samples were collected from five different locations and pooled to obtain one composite sample per pond. Microbiological analysis of the composite samples yielded a total of 103 colonies on Chromogenic Coliform Agar at a dilution factor of 10^{-7} (Table 2). From Pond 1, a total of 55 colonies were recovered. Among these, 15 colonies appeared dark blue to violet, presumptively indicating *Escherichia coli*, while 17 colonies appeared pink to red, representing other coliform bacteria. In addition, 11 colonies displayed salmon to red pigmentation, whereas 12 colonies appeared colourless or pale, suggesting non-coliform members of the *Enterobacteriaceae*.

Similarly, 48 colonies were recovered from Pond 2. Of these, 11 colonies were dark blue to violet (presumptive *E. coli*), 12 colonies were pink to red (other coliform bacteria), 15 colonies appeared salmon to red, and 10 colonies were colourless or pale, consistent with non-coliform *Enterobacteriaceae*. Overall, the chromogenic reactions observed across both ponds indicate the presence and distribution of different *Enterobacteriaceae* groups in the earthen pond water, with coliform bacteria representing the predominant colony types.

Table 1: PCR primers used in this study

Target	Primer sequence (5'–3')	Annealing temp (°C)	Product size (bp)
tetA	F: GCTACATCCTGCTTGCCTTC	55	210
	R: CATAGATCGCCGTGAAGAGG		
tetM	F: CGAGGTCCGTCTGAACTTTGC	55	583
	R: GCGGCACTTCGATGTGAATGGT		

Table 2: Chromogenic colony distribution of *Enterobacteriaceae* isolates from earthen catfish pond water

Pond ID	Number of sampling points	Composite samples collected	*Number of colonies	Colony type
Pond 1	5	1	55	15 dark blue/violet, 17 pink/red, 11 Salmon to red, 12 colourless/pale ((non-coliform)
Pond 2	5	1	48	11 dark blue/violet, 12 pink/red, 15 Salmon to red, 10 colourless/pale ((non-coliform)

*Colonies recovered from dilution factor of 10^{-7}

Table 3: Mean zone of inhibition and antibiotic sensitivity pattern of the selected bacterial isolates

Antibiotics	Mean Zones of inhibition (mm) ± SD			
	EP1	EP2	EP3	EP9
Ciprofloxacin (5 µg)	30.4 ± 1.3 (S)	24.5 ± 1.9 (S)	25.2 ± 1.1 (S)	32.4 ± 1.6 (S)
Ampicillin (10 µg)	0.0 ± 0.0 (R)	0.0 ± 0.0 (R)	0.0 ± 0.0 (R)	0.0 ± 0.0 (R)
Tetracycline (30 µg)	15.4 ± 1.4 (I)	16.3 ± 1.2 (I)	12.4 ± 1.2 (I)	0.0 ± 0.0 (R)
Cefuroxime (30 µg)	0.0 ± 0.0 (R)	0.0 ± 0.0 (R)	0.0 ± 0.0 (R)	0.0 ± 0.0 (R)

Values are presented as mean ± standard deviation of triplicate determinations (n = 3). Antibiotic susceptibility interpretation was performed according to the Clinical and Laboratory Standards Institute (CLSI). Key: S = Sensitive; I = Intermediate; R = Resistant.

Antibiotic susceptibility of isolates:

The antibiotic susceptibility of the four selected bacterial isolates (EP1, EP2, EP3, and EP9), as shown in Table 3, indicated significant differences in their responses to the tested antibiotics. All the four isolates were sensitive to ciprofloxacin with isolate EP9 having highest mean zone diameter of inhibition and isolate EP2 with the lowest.

All four isolates, however, were resistant to ampicillin and cefuroxime, as shown by the absence of inhibitory zones. Isolate EP9 was resistant to tetracycline with no zone of inhibition, while isolates EP1, EP2, and EP3 showed intermediate sensitivity to tetracycline.

Culture isolation and biochemical identification of representative *Enterobacteriaceae*:

The biochemical and sugar fermentation profiles of the four representative isolates are presented in Table 4. The motility test for the isolate designated EP9 did not show visible growth radiating from the point of inoculation, unlike the other isolates, and was therefore considered negative.

Table 4: Sugar fermentation and biochemical characteristics of selected bacterial isolates from earthen catfish ponds

Test	EP1	EP2	EP3	EP9
Gram reaction	– (rod)	– (rod)	– (rod)	– (rod)
Oxidase	+	–	–	–
Catalase	+	+	+	+
Motility	+	+	+	–
Indole	–	+	–	+
Citrate utilization	–	–	+	–
Urease	–	–	–	–
Methyl red	+	+	+	+
Voges–Proskauer	–	–	–	–
TSI (slant/butt)	A/A	A/A	K/A + H_2S	A/A
Gas production	+	+	–	–
H_2S production	–	–	+	–
Sugar fermentation				
Glucose	+	+	+	+
Lactose	–	+	–	–
Sucrose	+	+	–	–
Mannitol fermentation	+	+	+	+
Presumptive identity	*Aeromonas* spp.	*Escherichia coli*	*Salmonella* spp.	*Escherichia* spp

Key: A/A = Acid slant / Acid butt (sugar fermentation); **K/A** = Alkaline slant / Acid butt; **+** = Positive reaction; **–** = Negative reaction **H_2S** = Hydrogen sulphide production

Molecular identification and phylogenetic relationship of the tetracycline-resistant isolate: The isolate designated EP9 was identified based on the highest sequence similarity and alignment score with reference sequences in the database. BLAST analysis revealed that isolate EP9 showed the closest phylogenetic affiliation with *Escherichia albertii*. The 16S rRNA gene sequence of isolate EP9 has been deposited in the NCBI nucleotide sequence database (GenBank) under the accession number PX312839.1.

The resulting phylogenetic tree (Fig. 2) resolved the analyzed sequences into two well-defined major clades corresponding to *Escherichia albertii* and *Escherichia coli* lineages. The Maximum Likelihood phylogenetic tree showed that isolate EP9 PX312839.1 clustered within the *Escherichia albertii* clade, grouping closely with reference *E. albertii* strains and clearly separated from *Escherichia coli* sequences. This confirms the taxonomic placement of the isolate as *Escherichia albertii*.

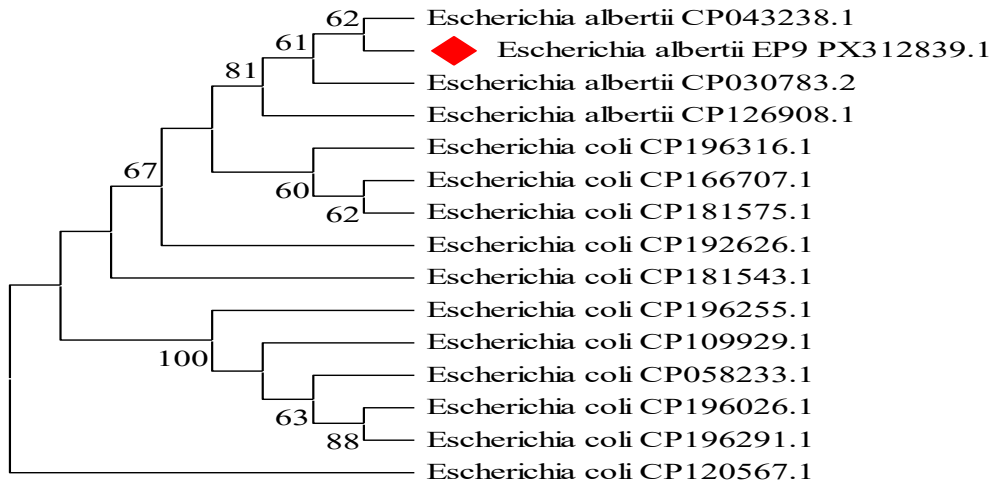

Fig 1: Phylogenetic tree illustrating the evolutionary relationship between the *Escherichia albertii* EP9 sequence identified in this study and closely related *Escherichia* spp. sequences retrieved from the NCBI GenBank database.

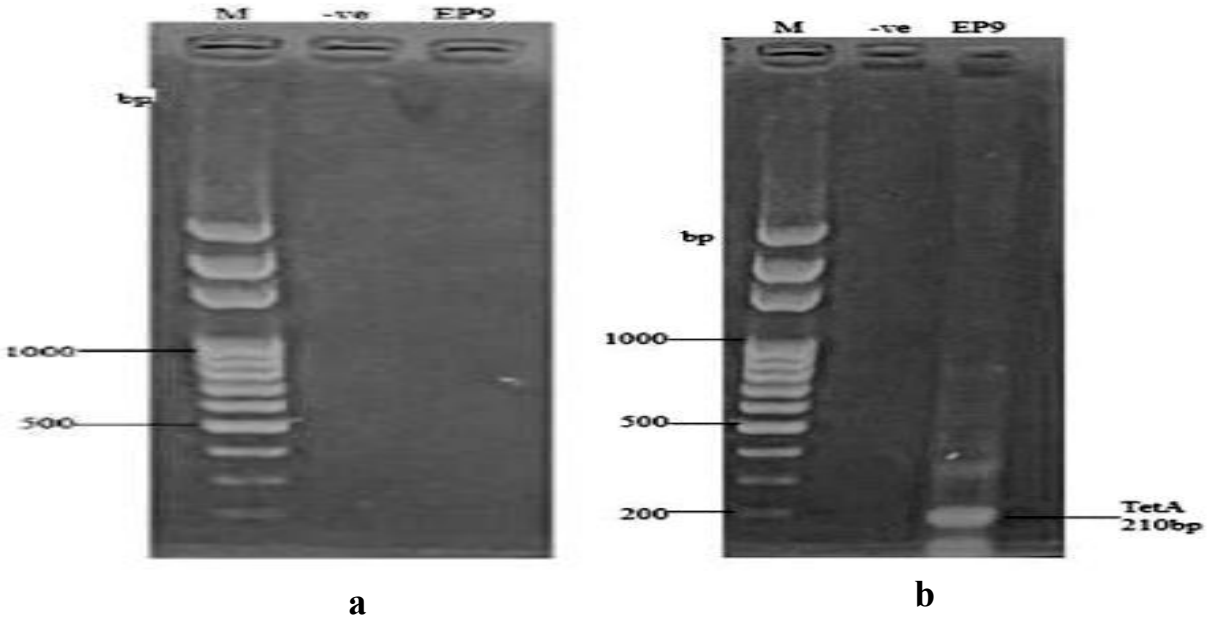

Fig 2: Agarose gel electrophoresis of PCR products for tetracycline resistance genes. M: DNA marker; –ve: negative control; EP9: test isolate. **(a)** shows absence of the *tetM* gene (583 bp), **(b)** shows presence of the *tetA* gene (210 bp)

PCR detection of tetracycline resistance genes:

Agarose gel electrophoresis of the PCR amplified products showed no detectable amplicon corresponding to the *tetM* gene, indicating a negative result for this determinant. In contrast, PCR amplification targeting the *tetA* gene produced a clear and distinct band at approximately 210 bp, consistent with the expected amplicon size for *tetA* (Fig. 2). These results confirm the presence of the *tetA* gene and the absence of *tetM* in the tested isolate.

Discussion:

The emergence and dissemination of antimicrobial resistance (AMR) in environmental and clinical bacteria constitute a major global public health concern. In this study, a tetracycline-resistant isolate (EP9) was recovered from an earthen fish pond in Akure, Ondo State, Nigeria, an aquaculture setting recognized as a potential hotspot for resistant bacteria due to routine antibiotic application. Molecular analysis identified the isolate as *Escherichia albertii*, an emerging zoonotic pathogen.

The antibiotic susceptibility profile of isolate EP9 reveals a classical multidrug-resistant (MDR) phenotype, defined by resistance to three or more antimicrobial classes (10), which in this case included ampicillin, cefuroxime and tetracycline. Complete resistance to ampicillin and cefuroxime, alongside acquired tetracycline resistance, mirrors patterns commonly reported among members of the family *Enterobacteriaceae* from aquatic and environmental sources (11,12). The universal resistance to ampicillin (a penicillin) and cefuroxime (a second-generation cephalosporin) strongly suggests the production of broad-spectrum β-lactamases, most plausibly TEM-1 or SHV-1 enzymes, which remain among the most prevalent β-lactamases in *Enterobacteriaceae* worldwide (13,14).

In contrast, the retained susceptibility of EP9 to ciprofloxacin, a fluoroquinolone, is notable but potentially transient. Fluoroquinolone resistance is increasingly mediated by plasmid-encoded determinants such as *qnr* genes, *aac(6′)-Ib-cr*, and efflux systems, which often co-localize with β-lactam and tetracycline resistance genes on mobile genetic elements (15, 16). The coexistence of ciprofloxacin susceptibility with an otherwise MDR phenotype may therefore represent an intermediate evolutionary stage preceding the acquisition of full fluoroquinolone resistance. This observation reinforces the need for sustained AMR surveillance and prudent fluoroquinolone use in both clinical and agricultural contexts to preserve the efficacy of this critical antimicrobial class (17,18).

A key strength of this study lies in the precise taxonomic resolution of the tetracycline-resistant isolate. Using 16S rRNA gene sequencing and robust phylogenetic analysis, EP9 (accession number PX312839.1) clustered within a distinct monophyletic *E. albertii* clade, clearly separated from *E. coli*. This distinction elevates the significance of the findings beyond the commonly studied *E. coli* framework. Although *E. albertii* is increasingly recognized as an emerging enteropathogen responsible for human gastrointestinal disease (19,20), its role in environmental reservoirs of antimicrobial resistance remains poorly defined. Traditionally, *E. albertii* has been associated with clinical and foodborne contexts, but recent genomic investigations have begun to uncover its presence beyond clinical settings. A recent study reported the first genomic characterization of multidrug-resistant *E. albertii* isolated from retail fish, highlighting its occurrence in aquaculture-associated food sources and raising concerns about fish as a vector for the dissemination of pathogenic and resistant strains (21). The isolate exhibited phenotypic resistance to multiple antibiotics and harboured mobile genetic elements such as plasmids and transposons that facilitate gene exchange, suggesting potential for ARG transfer in food-related environments.

In addition to aquatic food sources, other studies have documented widespread multidrug resistance and ARG carriage in *E. albertii* from non-aquatic food products; for instance, poultry meat isolates exhibited high levels of resistance to clinically relevant antibiotics and harboured corresponding resistance genes identified by whole-genome sequencing (22). Older work on *E. albertii* from clinical and environmental sources similarly observed resistance to tetracycline and other antibiotics, indicating that this species is capable of acquiring and maintaining diverse resistance determinants (23). Collectively, these findings support the notion that *E. albertii*, like other members of *Enterobacteriaceae*, is not only a potential zoonotic pathogen but also a carrier of ARGs, including determinants for tetracyclines, β-lactams and other antimicrobial classes.

Molecular analysis identified the *tetA* gene as the detected determinant associated with tetracycline resistance in *E. albertii* EP9, while *tetM* was not observed. This resistance profile is consistent with the farmer's reported reliance on oxytetracycline as the primary anti-

microbial used in the pond, suggesting antibiotic-driven selection pressure in the aquaculture environment. Although the present analysis was limited to a subset of tetracycline resistance genes and does not exclude the possible presence of additional *tet* determinants, the targeted detection of *tetA*, one of the most prevalent and epidemiologically relevant tetracycline resistance genes in aquatic systems, represents a key strength of the study. Previous studies have shown that aquaculture systems with a history of oxytetracycline application exhibit significantly higher detection frequencies of tetracycline resistance genes, particularly *tetA,* than facilities without recent tetracycline exposure (5), underscoring the strong selective pressure exerted by antibiotic use in such environments. The *tetA* gene is a plasmid-encoded efflux pump that confers significant resistance to tetracycline. This gene, along with *tetB* are the most prevalent among tetracycline-resistant *Enterobacteriaceae* (24), allowing promiscuous dissemination between cells, often alongside other resistance genes, and contributing significantly to antibiotic resistance.

Within the context of this study conducted in Akure, the detection of *tetA* in the absence of *tetM* likely reflects both sustained oxytetracycline-driven selection and the ecological characteristics of earthen pond systems. These environments promote close interactions between bacterial communities in water and sediments, thereby facilitating horizontal gene transfer of mobile resistance determinants, especially plasmid-borne genes such as *tetA*. Notably, previous reports have emphasized that aquaculture settings can support the persistence and dissemination of antibiotic resistance genes even in the absence of detectable antibiotic residues, owing to the stability of environmental resistance gene pools and continuous microbial exchange (10).

Globally, investigations of freshwater aquaculture systems consistently identify tetracycline resistance genes among the most prevalent AMR determinants. A meta-analysis has revealed strong association between environmental tetracycline concentrations and the abundance of *tet* genes, including *tetA*, reinforcing the role of ponds as long-term reservoirs of resistance elements (25). Similarly, studies from commercial fin-fish culture systems in India reported *tetA* as the dominant resistance gene among heterotrophic bacteria isolated from water, sediment, and fish tissues, further supporting the widespread distribution of this efflux-mediated resistance mechanism in aquaculture settings subjected to tetracycline exposure (26). Earthen aquaculture ponds provide conditions conducive to the persistence and horizontal dissemination of resistance genes, owing to high organic load, dense microbial populations, and sustained environmental exposure.

The *tetA* gene encodes an energy-dependent efflux pump that actively exports tetracycline from the bacterial cell, thereby reducing intracellular drug accumulation. This gene is typically plasmid-borne, facilitating horizontal gene transfer among diverse bacterial taxa. In contrast, *tetM* encodes a ribosomal protection protein commonly associated with conjugative transposons. The absence of *tetM* in EP9 suggests selective enrichment of plasmid-mediated efflux mechanisms within this ecological niche, potentially driven by co-selection with other plasmid-encoded resistance determinants. Collectively, these findings indicate that aquaculture environments not only select for tetracycline resistance but also favour genetic platforms that enhance the dissemination of multidrug resistance within environmental bacterial communities.

Conclusion:

This study demonstrates that earthen catfish ponds in Akure, Nigeria, serve as environmental reservoirs of multidrug-resistant *Enterobacteriaceae* and harbour clinically relevant tetracycline resistance determinants. The recovery and molecular characterization of a tetracycline-resistant *E. albertii* isolate carrying the plasmid-mediated *tetA* gene highlight the expanding ecological niche of this emerging zoonotic pathogen beyond clinical and foodborne settings into aquaculture environments. The absence of *tetM* alongside the presence of *tetA* suggests selective enrichment of efflux-mediated resistance mechanisms, likely driven by sustained oxytetracycline use and facilitated by horizontal gene transfer within pond microbial communities.

The detection of multidrug resistance in an aquaculture-associated *E. albertii* strain raises important public health concerns, given the potential for transmission through fish consumption, occupational exposure, and environmental dissemination. These findings reinforce the role of aquaculture systems as interfaces linking environmental, animal, and human reservoirs of antimicrobial resistance. Collectively, this work provides molecular evidence that underscores the need for routine surveillance of resistance genes in aquaculture settings, stricter regulation of antibiotic use, and the adoption of alternative disease management strategies such as vaccination, probiotics, and improved farm hyg-

iene. Strengthening antimicrobial stewardship in aquaculture is essential to limit the persistence and spread of resistance determinants and to safeguard both food safety and public health.

Contribution of authors:

FOS, OP and SLB conceptualized and designed the study. FOS and OP were involved in data collection. FOS, OP and SLB were involved in the writing and revising the manuscript for intellectual content. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.

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Authors declare no conflict of interest.

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