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Prevalence of antimicrobial-resistant *Escherichia coli* in freshwater catfish (*Clarias gariepinus*) sampled in Nsukka Nigeria, and risk factors for its spread

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Abstract

Rapid expansion in aquaculture has exacerbated the threat of antimicrobial resistance (AMR) especially in sub-Saharan Africa, amid limited surveillance. This study determined the prevalence of antimicrobial resistant *Escherichia coli* in fresh water catfish (*Clarias gariepinus*) sampled in Nsukka Nigeria, and further evaluated risk factors associated with its spread, using a One Health approach. The study was a cross-sectional survey. A total of 265 swab samples comprising 150 from raw catfish and 115 from **h**andlers and contact surfaces were processed for *E. coli* identification using standard techniques. The AMR profile of the isolates were assessed using both disc diffusion and agar dilution methods, while the associated risk factors among fish farmers and processors (n = 150) were assessed with questionnaires. Results showed that the prevalence of *E. coli* was 11.3% in the sampled catfish, with *E. coli* contamination rates of 24.3%, 8.6% and 4.7% at restaurants, markets, and fish ponds respectively. The prevalence in handlers' hands and contact surfaces were 11.4% and 11.2% respectively. The *E. coli* prevalence rates were significantly ($p < 0.05$) associated with the source of the fish (ponds, markets or restaurants/hotels). *E. coli* isolates resistant to antimicrobial agents were 13 (4.9%) of the 265 total samples. Forty percent of the isolates were resistant to erythromycin, while only 3% were resistant to colistin. All the isolates (100%) were sensitive to ciplofloxacin, gentamicin and ceftriaxone. Identified risk factors for the spread of antibiotic resistant *E. coli* in catfish and catfish handlers in Nsukka Nigeria included: Lack of AMR awareness (75 – 80%), use of antibiotics without prescription (60%), and non-adherence to antimicrobial withdrawal periods (60%). It was concluded that catfish in Nsukka Nigeria is contaminated with antimicrobial resistant *E. coli*, with post-harvest environments and handlers serving as critical hotspots for its spread, hence the need for urgent interventions, including awareness campaign on antimicrobial stewardship and improved food hygiene practices.

Keywords: *Escherichia coli*; Prevalence; Catfish; Nsukka Nigeria; Antimicrobial resistance; One Health.

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Introduction

Antimicrobial resistance (AMR) is a critical threat to global public health, with high burden in developing countries and Africa as a continent (Osena *et al*., 2025). Compounding this issue is the region's rapidly growing aquaculture sector, where many farmers are expanding into catfish farming but operate with limited AMR surveillance. This creates a potential amplifier opportunity for spread of antimicrobial resistant bacteria in the industry that supplies over 28% of fish and 3.26% of agricultural gross domestic product with annual per capita fish consumption in Africa reaching 10.8 kg in Africa (Reverter *et al*., 2020; WHO, 2020; Okon *et al*., 2022; FAO, 2024). In Nigeria, aquaculture/fish production contributes significantly to the economy, generating approximately $848.6 million in revenue (FAO, 2024; Okon *et al*., 2025). However, the increasing protein demands and corresponding shift from semi-intensive to intensive fish production systems may have exacerbated AMR emergence in the country (Manyi-Loh *et al*., 2018).

As a crucial indicator for AMR monitoring, the pathogenic and resistant strains of *E. coli* species pose a dual threat as they cause significant economic losses in aquaculture through fish death and spoilage, and also present a serious risk of difficult-to-treat zoonotic infections in humans (Watts *et al*., 2017; Jeamsripong *et al.*, 2023). Of particular concern are extended-spectrum β-lactamase (ESBL)-producing *E. coli* strains, which hydrolyze penicillins, cephalosporins and even last-resort drugs like colistin, thereby severely limiting treatment options for severe infections (Sousa *et al*., 2011; Cheng *et al*., 2014). The transmission of resistant *E. coli* through fish consumption or handler contact poses direct risks to human health (Anyanwu and Chah, 2016; Van *et al*., 2020). Moreover, the high level of human-animal-environment interaction within aquaculture environment including fish market, restaurants and fish ponds, coupled with many risk factors including high stocking densities in fish ponds, inadequate hygiene practices, inappropriate use of antibiotics and poor waste management may have presented them as potential hotspots for the spread of multi-drug resistant (MDR) pathogens in Nigeria (Alonso *et al*., 2017; Thornber *et al*., 2020; Velazquez-meza *et al*., 2022).

African catfish (*Clarias gariepinus*) farming has become a popular agricultural business sector in Nigeria, as reportedly, the species can adapt to a wide range of temperatures, tolerates low oxygen and low salinity levels, and can mature in about six months (Adetola, 2023). Nigeria is now among the largest producer of catfish in the world, and the livelihoods of millions depend on it for the provision of lean protein and energy. It helps maintain healthy bones and tissue repair, among other benefits. No wonder it has become a delicacy, especially among the elderly. Despite being one of the highest producers of catfish, Nigeria is still struggling to bridge the gap between the estimated annual demands of 3.6 million metric tonnes against the national domestic fish supply estimated at 1.2 million metric tonnes (Adetola, 2023).

Antimicrobial resistance issues have been compounded by the fact that 60 – 80% of Nigerian fish farmers reportedly use antibiotics without veterinary oversight, and often disregarding withdrawal periods (Dandi *et al*., 2024). Such practices accelerate resistance development and may lead to treatment failures in both aquaculture and human medicine (Laxminarayn *et al*., 2016). A study in Western Nigeria has documented antimicrobial resistant *E. coli* prevalence rates of 17.5% in catfish (Akande and Onyedibe, 2019). However, data specific to Nsukka aquaculture sector in South-east Nigeria on AMR in fish is scarce. Currently, there is a significant knowledge gap regarding the risk factors that drive the emergence and transmission of antimicrobial resistant *E. coli* within the aquaculture value chain of Nigeria. Local studies are reportedly indispensable for generating the specific, real-time data needed to understand the scope and patterns of AMR in the country (WHO, 2022; Velazquez-meza *et al*., 2022). The present study determined the prevalence of antimicrobial-resistant *E. coli* in freshwater catfish in Nsukka Nigeria, and further evaluated risk factors associated with its spread, using a One Health approach.

Materials and Methods

Study area: The study was conducted in Nsukka, Enugu State, Nigeria (6°18′ – 7°00′N, 7°52′ – 7°54′E) from December 2024 to February 2025. Nsukka shares boundaries with Igbo-Etiti to the west, Udenu to the east, and Igbo-Eze to the north (Figure 1), in Enugu State, Nigeria, and serves as a critical contact zone between the northern and southern regions of Nigeria, with neighboring Idoma (Benue State) to the northeast, Igala (Kogi State) to the north and northwest, Ishielu (Ebonyi State) and Nike (Enugu State) to the east, and Udi (Enugu State) and Ifite Awgwali (Anambra State) to the south. The inhabitants of Nsukka are primarily civil servants, students, traders, healthcare workers and farmers specializing in piggery and poultry production. The area now hosts high-density fish farms, markets and restaurants, which serve as critical nodes in the aquaculture value chain (Akande and Onyedibe, 2019).

Ethical considerations: Ethical Approval for the study was obtained from the Institutional Animal Care and Use Committee of the University of Nigeria, Nsukka (Approval Reference Number: FVM-UNN-IACUC-2025-03/219). Informed consent was obtained from all human participants, and confidentiality of their data was maintained throughout the study.

Study design: The study was a cross-sectional quantitative and qualitative survey conducted between December 2024 and February 2025, on fish samples collected from major hotels, restaurants, markets and fish ponds in Nsukka, using systematic random sampling. Swabs were collected from the hands of consenting fish handlers and contact surfaces. Questionnaire was used to collect information from fish handlers in the study area.

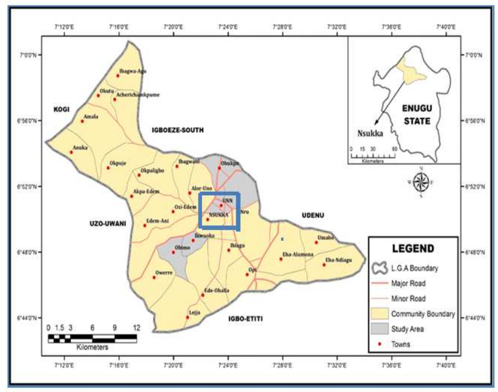

Figure 1. The map of Nsukka (the study area), with the blue square indicating the sample collection site at Nsukka Metropolis, Nigeria.

Sample size determination: The minimum sample size for this study was calculated using the formula n= Z^2×P× (1-P)/d2 (Thrusfield, 2005), at 95% confidence level and a 5% margin of error, with a known prevalence of *E. coli* 17.5% (Akande and Onyedibe, 2019). A minimum sample size of 222 was calculated; however a total of 265 samples (150 fish, 35 handlers, 80 surfaces) were collected to improve statistical power.

The 265 samples collected comprised: Fish: 43 from ponds, 70 from markets, 37 from restaurants/hotels; Handlers: 35 hand swabs and Contact surfaces: 80 swabs of knives and tables. The samples (fish skin, gills, intestines, handlers' hands and surfaces) were transported aseptically on ice packs within two hours to the Department of Veterinary Public Health and Preventive Medicine Laboratory, University of Nigeria, Nsukka, for analysis.

Isolation and identification of *E. coli*: Intestinal samples were homogenized in sterile phosphate-buffered saline and incubated at 37°C for 24 hours in buffered peptone water. Swab samples were transferred to tubes containing 4 ml buffered peptone water, incubated aerobically at 37°C for 24 hours, and cultured on MacConkey agar (Oxoid, England). Lactose-fermenting colonies (pinkish growth) were sub-cultured on Eosin Methylene Blue agar (EMB; Oxoid, England). Colonies exhibiting a metallic green sheen were tentatively identified as *E. coli* spp. They were subjected to Gram staining and biochemical tests (indole, citrate utilization, and triple sugar iron agar tests) and further confirmed using PCR techniques as previously described by Nwankwo *et al*. (2025), then, stored at 4°C on nutrient agar slants for antimicrobial susceptibility testing.

Detection of extended-spectrum beta-lactamase (ESBL) producers: ESBL production was assessed using the agar dilution method as described (CLSI, 2025). Ceftriaxone (0.05 mg/mL) was incorporated into EMB agar, and isolates were streaked after adjusting to 0.5 McFarland standard. Plates were incubated at 37°C for 24 hours. Growth indicated potential ESBL production.

Antimicrobial susceptibility testing: Five antibiotics commonly used by farmers in the study area namely; Ciprofloxacin (CIP) 5µg, Gentamicin (GEN) 10µg, Erythromycin (E) 5µg, Ceftriaxone 30 µg and Colistin sulphate (Mast Disc Ltd, UK) belonging to five different classes from the lists of critically important antimicrobials for humans and animals were used (WHO/FAO, 2021). Disc diffusion (Mueller-Hinton agar) test was performed following CLSI (2025) guidelines. Isolates were classified as sensitive or resistant (intermediate results were grouped as sensitive).

Questionnaire survey: Literature and expert opinions were objectively used to design a well-structured questionnaire with mostly close-ended questions. The questionnaire contained sections on fish farmers' demographic characteristics including their age, education, farming experience, knowledge, attitude and practices including antibiotic use, sources, adherence to withdrawal periods and AMR awareness. Fish farmers and processors that consented to participate in the study were interviewed at the point of sample collection in their individual farms with the questionnaire. In some cases the questions were verbally interpreted in the local (Igbo) language for the respondent's comprehension. Assurances, confidentiality, and opportunity to withdraw at any time without prejudice were maintained with the respondents throughout the study in line with the Helsinki Declaration (WMADH, 2001).

Data analysis: Data were subjected to descriptive statistics using SPSS (version 27). Associations between prevalence and sample sources were tested with Chi-square or Fisher's exact tests, as appropriate. Significance was accepted at $p < 0.05$.

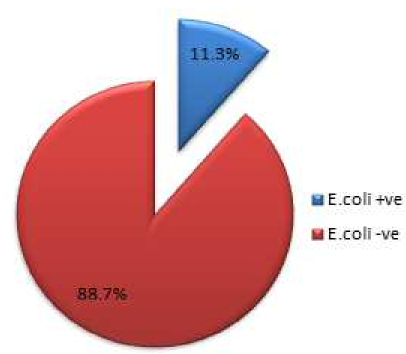

Figure 2. Overall prevalence of *E. coli* in fish, contact surfaces and handlers in Nsukka, Enugu State, Nigeria.

Results

Escherichia coli was isolated from 30 out of the 265 samples (11.3%) collected for the study (Figure 2). The distribution as per sample sources were: 9/37 (24.3%) samples from restaurants/hotels; 6/70 (8.6%) samples from fish markets; and 2/43 (4.7%) samples from fish ponds (Table 1). For the contact surfaces, the prevalence distribution was: 4/35 (11.4%) fish handlers' hands; and 9/80 (11.2%) contact surfaces (tables and knifes) [Table 2]. The prevalence of *E. coli* was significantly (p = 0.0132) associated with the fish sample sources (Table 1), but there was no significant (p = 0.9714) association between the prevalence and distribution on fish handlers/contact surfaces (Table 2).

The prevalence of ESBL *E. coli* strains (resistant to ceftriaxone) was zero (0%). On further evaluation of the antimicrobial resistance profile of the 30 *E. coli* isolates identified, those resistant to the antimicrobial agents were 13 (4.9%) out of the total 265 samples; 5/37 (13.5%) samples from restaurants/hotel; 2/70 (2.9%) samples from fish market; 1/43 (2.3%) samples from fish pond; 3/35 (8.6%) samples from fish handlers; and 2/80 (2.5%) samples from fish contact surfaces (Figures 3 and 4). 3.3% of the samples were resistant to colistin, while 40% were resistant to erythromycin (Figure 5). All the isolates (100%) were sensitive (resistance = 0%) to gentamicin and ciprofloxacin (Figure 5).

Table 1. Prevalence of *E. coli* in fresh water catfish for human consumption sampled in Nsukka, Enugu State, Nigeria

Fish sources	Total number of fish samples collected.	Total number positive for *E. coli*, with % in bracket	ESBL producers
Fish ponds	43	2 (4.7%)	0
Fish market	70	6 (8.6%)	0
Restaurants/Hotel	37	9 (24.3%)	0
Total	150	17 (11.3%)	0

$\chi 2 = 8.656$; p = 0.0132; ESBL - Extended-spectrum beta-lactamase

Table 2. Prevalence of *E. coli* in fish handlers' hands and contact surfaces sampled in Nsukka, Enugu State, Nigeria

Sample source	Total number of swab samples	Total number positive, with % in bracket	ESBL producers
Fish handlers' hands	35	4 (11.4)	0
Fish contact surfaces (tables and knives)	80	9 (11.2)	0
Total	115	13 (8.7)	0

P = 0.9714; ESBL - Extended-spectrum beta-lactamase

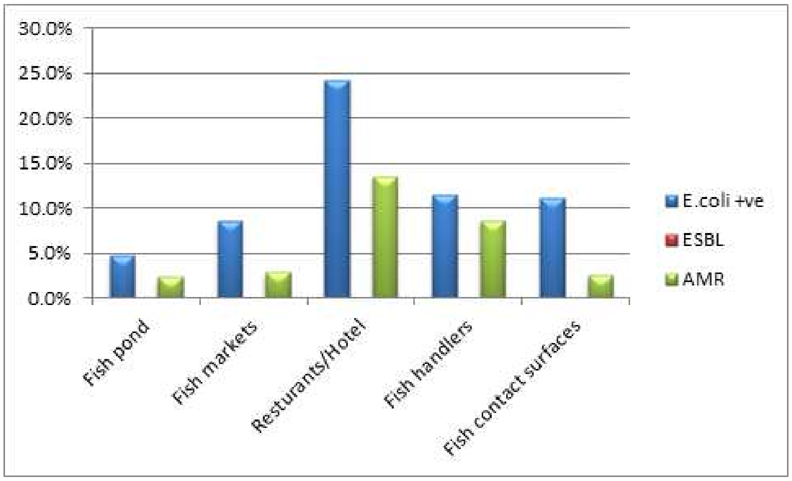

Figure 3. Prevalence of *E. coli* and antimicrobial resistant species in fish from different sources, handlers and fish contact surfaces in Nsukka Metropolis, Nigeria.

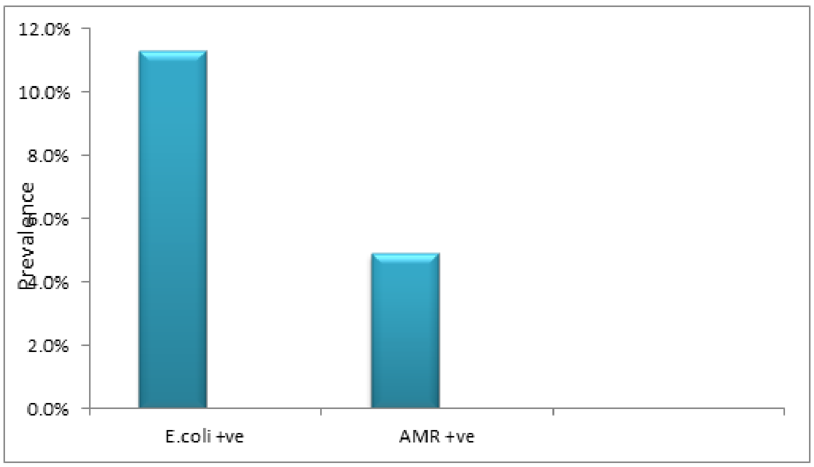

Figure 4. Overall prevalence of *E. coli* and AMR species in fish of different sources, handlers and fish contact surfaces in Nsukka Metropolis, Nigeria.

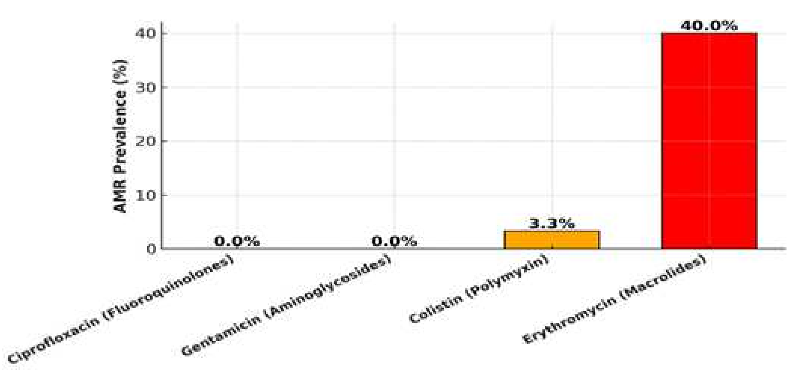

Figure 5. Antimicrobial resistance profile of *E. coli* isolates from fish, handlers and contact surfaces in Nsukka, Enugu State, Nigeria.

The socio-demographic characteristics of the respondents to the questionnaire revealed that majority of the respondents were males (62.5%) while females were 37.5% (Figure 6). With respect to age, young adults of 20 – 40 years constituted 87.5% of the respondents, while those of 50 – 65 years age groups made up 12.5% (Figure 6). Fifty percent of the respondents had education up to the secondary school level, and those that had only primary school education were 37.5%, while those with tertiary education were 12.5% (Figure 6). Equal percentages of the farmers has been experienced in the business for more than 10 years (50%) or had 5 – 10 years of experience (50%) [Figure 6].

On the use of antimicrobials in the farm, 75% of the respondents reported the use of antimicrobials while 25% reported not using antimicrobials at all. Majority (55%) of those that use antimicrobials source them from other big farmers while the least (20%) buy over the counter from medicine shops/pharmacy outlets. Majority (53%) of the farmers prefer antimicrobials already in combination therapy mostly for the purpose of disease treatment (50%), disease prevention (30%) and as growth promoter (20%). The application of antimicrobials by the farmers was reported to be done 'regularly' by 20% of the respondents, 'rarely' by 30%, and 'occasionally' by 50% (Figure 7).

On the awareness of AMR, health and hygiene safety amongst the fish farmers, 75% of them lack knowledge of AMR while the 25% with the knowledge acquired it majorly (58%) from health professionals. Furthermore, 80% of the farmers have no knowledge about ESBL or use third generation cephalosporins in their farms. Most of the respondents (60%) do not consult veterinarians for diagnosis and proper prescription of the antimicrobials they use in their farms of which they acquired majorly from non-veterinary sources (50%) [Figure 7].

Over 60% of the respondents do not observe antibiotics withdrawal periods before selling or distribution of fish to the public, while 45% of the respondents practice mixed farming. Although majority of these farmers (63%) do not share farm equipment and tools between their farms, a good number admitted of engaging in such practices (37%). Furthermore, majority (60%) of the farmers reported that they experience abnormal fish mortality and when death occurs, most of them (40%) dispose of them via burying, and 30% use the dead fish in feeding the living fish, while 30% dispose of the dead in open environment (Figure 7).

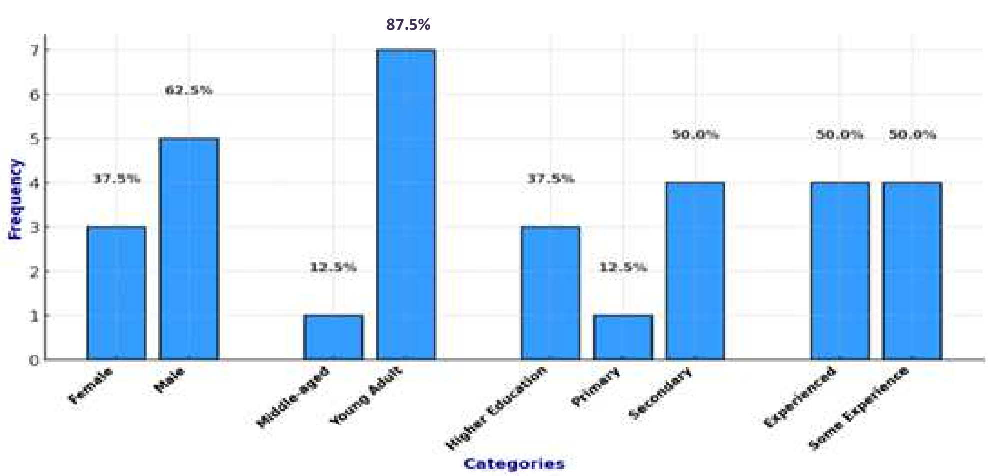

Figure 6. Socio-demographic characteristics of the fish farmers surveyed within Nsukka Metropolis, Nigeria.

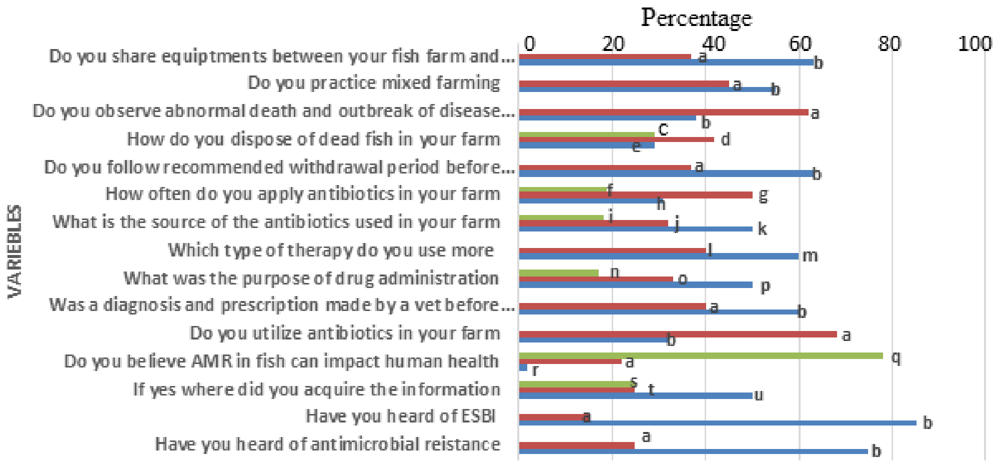

Figure 7. Knowledge, attitude and risk practices associated with AMR spread amongst fish farmers in Nsukka, Enugu State, Nigeria.

Key: a = Yes, b = No, c = feeding of other fish, d = burying, e = open disposal, f = regularly/weekly, g = occasionally/monthly, h = rarely/yearly, i = vets, j = pharmacy, k = other farmers, l = single, m = combination, n = growth enhancement, o = prevention, p = treatment, q = unsure, r = no idea, s = colleagues and friends, t = media, u = health professionals.

Discussion

The 11.3% overall prevalence of *E. coli* in the catfish samples and contact surfaces surveyed and the 24.3% recorded at restaurants/hotels is thought to probably be due to poor handling and processing hygiene, especially at restaurant or the last point of human contacts before the consumers' table. This is a pointer to a high risk of infection transmission and AMR spread to processors and consumers of contaminated catfish in Nsukka, Nigeria. Moreover, the 11.4% and 11.2% prevalence among the fish handlers and contact surfaces respectively, confirms that these fish handlers and contact surfaces are critical hazard analysis control points for intervention against the spread of *E. coli* in the aquaculture food chain (Nwankwo and Ntaudom, 2025). Control measures should therefore, target not only judicious use of antimicrobials in catfish farming and hygienic handling during harvesting at farms, but at sales and processing points in the markets and public eating places. Meanwhile, market environment has been reported as point of antimicrobial resistant spread even for poultry products (Nwankwo *et al*., 2025). Adequate cleaning and use of disinfectants on tables and knives before and after each batch of fish sales and processing should be encouraged (Bedane *et al*., 2022; Dewi *et al.*, 2022)

The 8.6% prevalence of *E. coli* contamination in catfish sourced at Nsukka fish market in the present study was lower than 17.5% reported in catfish at fish markets in Jos, Nigeria (Akande and Onyedibe 2019). It however mirrors the findings by Sudaryatma *et al*. (2025), which identified fish markets as critical AMR transmission nodes. The variations in the prevalence may not be entirely attributed to the differences in water quality but other environmental conditions as well as hygiene practices across these geographic regions, hence reinforcing the need for improved hygienic practices among fish farmers and processors in the country.

Since *E. coli* is not a natural commensal of fish, its detection in farmed fish is of public health concern, even though the lowest prevalence (4.7%) was at the fish ponds. The prevalence recorded in the present study in fish ponds

(4.7%) was very low compared to 44.5% reported in farmed fish in Malaysia (Dewi *et al*., 2022). Notably, the low farm-level prevalence (4.7%) contrasts with reports from intensive systems in Bangladesh (Afrose *et al*., 2025), possibly reflecting lower stocking densities or limited antibiotic use in Nsukka's small-scale farms. The differences may also be associated with the level of faecal contamination, likely stemming from poor waste management practices especially in mixed farming environment in addition to scale of production in the specific study areas. The practice of mixed farming, where fish farmers also rear animals such as pigs, goats, and sheep, and the sharing of farming equipment increase the risk of cross-contamination.

The higher *E. coli* contamination rate at the restaurants sampled aligns with studies from Malaysia (Dewi *et al*., 2022), where poor hygiene during food preparation have been reported to amplify the contamination rates. A review reported that the differences could be associated with regional factors including the environment (Alexanndre *et al*., 2025).

The overall antimicrobial resistant *E. coli* prevalence of 4.9% in this study was low compared to the range of 34.1 – 58.8% reported in other food producing animals in Lagos, Nigeria (Adenipekum *et al*., 2015), indicating potential variations in different regions, sources and antimicrobial usage in the AMR development. The highest prevalence AMR in hotels and restaurants (13.5%), followed by markets (2.9%) and fish farms (2.3%), highlight the critical role of food service establishments in the dissemination of AMR strains of food borne pathogens and the potential for transfer of resistant genes which could compromise treatment of infections in humans.

The report of 40% resistance of the *E. coli* isolates to erythromycin could be linked to its prophylactic use in aquaculture (Cheng *et al*., 2014), even though this is lower that 81.25% and 72.7% reported in fish from Bangladesh (Reza *et al*., 2020). The 3.3% resistance to colistin, though low, warrants vigilance given its status as a last-resort antibiotic (El-sayed *et al*., 2020). Further studies on pond water and sediment should be considered since there have been reports of multi-drug resistance compared to isolates from fish tissues. This finding underscores the need for consideration of the aquatic environment in the spread of AMR (Suyamud *et al*., 2024)

The absence of ESBL-producing *E. coli* in the present study suggests low circulation of ESBL genes in cultured cat fish unlike the report of 62% prevalence in raw mackerel sold in open market in Lagos, Nigeria (Okunye *et al*., 2022) as well as in other poultry studies (Thomber *et al*., 2020). This likely reflects limited use of third-generation cephalosporin (reported by 80% of farmers) and a less intensive human-livestock-fish interface compared to terrestrial farms (Alonso *et al*., 2017). However, these findings require validation with molecular methods to detect latent resistance genes (Jeamspring *et al*., 2023).

Encouragingly, all isolates showed full susceptibility to ceftriazone, gentamicin and ciprofloxacin, unlike the reported resistance except for ceftriazone in fish harvested from pond in Lagos Nigeria (Aina and Olaleye, 2023).This variations imply differences on the usage of antimicrobials in fish production in different geographical areas in Nigeria.

This study found a relatively lower male dominance (62.2%) of fish farming business compared to a report of 90% from Orlu, Imo State (Agbabiaka *et al*., 2025); a higher proportion of young adults (20 – 40 years); and a different educational profile, with 50% having attended secondary school versus 75% with tertiary education in the Imo State report, revealing different dynamics for fish farming and handling in the same region of the country. Furthermore, the questionnaires revealed some critical gaps: 75% lacked AMR awareness, consistent with Ghanaian aquaculture reports by Dandi *et al*. (2024); 60% used antibiotics without veterinary prescription, echoing patterns in Lagos (Laxmninarayn *et al*., 2016); and 45% practiced mixed farming, potentially facilitating zoonotic transmission (Watt *et al*., 2017). However, the spread could be minimal since 60% of them reported non-sharing of farm equipment between the farms. These findings agree with Dandi *et al*., (2024), where 52.75% of farmers relied on personal experience and 40.66% used antibiotics without prescription. Alarmingly, over 60% disregarded withdrawal periods, increasing risks of antimicrobial residues and resistant bacteria spread (Shallcvross and Davies, 2014; Aina and Olaleye 2023). With the record of high mortality rate by majority (60%) of the farmers, there is need for awareness on fish health and good maintenance of aquatic environment for the farmers rather than regular use of antimicrobials either for treatment (50%) or disease prevention (30%) as reported by 20% of the farmers. These align with WHO (2020) priorities for low-resource settings.

The contribution of health professionals in the control of AMR was revealed in the study as majority (60%) of the farmers acknowledged to have heard about AMR through health professionals. However, more regular training programmes are needed to discourage the use dead fish for feeding in addition to hygienic fish handling both at the farm level, fish seller's tables and among processors (Velazquez-meza *et al*., 2022). Further studies would encourage the use of molecular tools in detecting AMR genes in a wider range of geographic locations via One Health surveillance approach in Nigeria (Jeamsripong *et al*., 2023).

Conclusion: Catfish that are processed and sold for human consumption and the handlers in Nsukka are contaminated with antimicrobial resistant *E. coli*, with markets and restaurants serving as critical control points in the fish supply chain. Improved antimicrobial stewardship in fish farming, sustained AMR surveillance, coupled with awareness creation, mandatory observance of hygiene protocols at sales and processing points have become inevitable practices by all key stakeholders in aquaculture industry in Nigeria under a One Health framework.

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Declaration of Interests

The authors declare that there are no conflicts of interest related to this article.

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