

OCCURRENCE AND ANTIMICROBIAL RESISTANCE PATTERNS OF ENTEROBACTERIACEAE ISOLATED FROM COMPANION DOGS AND FISH POND WATER IN ADO-EKITI, NIGERIA

¹ADEWUMI, F. A., ²IPINLAYE, J. O. and ³OLUYEGE, A. O

^{1&3}Department of Medical Laboratory Science, Faculty of Basic Medical Science, Ekiti State University, Nigeria

²Department of Microbiology, Faculty of Science, Ekiti State University, Nigeria

³SDG 3 -Research Group³SDG 3

Abstract

*The emergence and dissemination of multidrug-resistant (MDR) Enterobacteriaceae constitute a growing public health threat within the One Health interface linking humans, animals, and the environment. This study investigated the occurrence, antimicrobial resistance profiles, and preliminary phenotypic carbapenem susceptibility of Enterobacteriaceae isolated from companion dogs and fish pond water in Ado-Ekiti, Nigeria. A total of 50 rectal swab samples from dogs and 25 fish pond water samples were aseptically collected and processed using standard microbiological methods. Isolates were identified based on colonial morphology, Gram staining, and biochemical characterization, while antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method according to Clinical and Laboratory Standards Institute guidelines. Preliminary phenotypic carbapenem susceptibility screening was conducted using imipenem and meropenem discs. Thirty-seven Enterobacteriaceae isolates were recovered, comprising *Klebsiella pneumoniae* (31/37, 83.8%) and *Escherichia coli* (6/37, 16.2%). Among dog isolates, *K. pneumoniae* accounted for 16/21 (76.1%) and *E. coli* for 5/21 (23.9%), while fish pond water samples yielded predominantly *K. pneumoniae* isolates (15/16, 93.7%). High resistance rates were observed among *K. pneumoniae* isolates against amoxicillin–clavulanate (87.5%), nitrofurantoin (81.3%), cefuroxime (68.8%), gentamicin (87.5%), levofloxacin (87.5%), and nalidixic acid (75.0%). Preliminary carbapenem screening revealed resistance to imipenem in 7/28 (25.0%) and meropenem in 2/28 (7.1%) of *K. pneumoniae* isolates, whereas *E. coli* isolates remained susceptible. The findings highlight companion animals and aquatic environments as important reservoirs of MDR Enterobacteriaceae and emphasize the need for strengthened surveillance, antimicrobial stewardship, and integrated One Health interventions.*

Keywords: Antimicrobial resistance; Multidrug-resistant Enterobacteriaceae; *Klebsiella pneumoniae*; *Escherichia coli*; Fish Pond water; Dogs; One Health

Introduction

The family Enterobacteriaceae comprises a diverse group of Gram-negative, facultatively anaerobic bacteria that are widely distributed in humans, animals, and environmental ecosystems. Among the most clinically important members are *Escherichia coli* and *Klebsiella pneumoniae*, which are major causes of urinary tract infections, septicemia, wound infections, pneumonia, and other opportunistic infections in both humans and animals (Pitout & Laupland, 2008; van Duin & Doi, 2017). These organisms are of significant public health concern because of their increasing ability to acquire and disseminate antimicrobial resistance determinants, leading to multidrug-

resistant infections that are difficult to treat.

Antimicrobial resistance (AMR) has emerged as one of the greatest global public health challenges of the twenty-first century. Recent estimates suggest that AMR contributed to nearly 4.95 million deaths worldwide in 2019, with resistant Gram-negative bacteria accounting for a substantial proportion of these deaths (Murray *et al.*, 2022). The rapid spread of resistant Enterobacteriaceae has reduced the effectiveness of commonly used antibiotics resulting in prolonged illness, increased healthcare costs, treatment failure and higher mortality rates. Resistance genes are often carried on plasmids and other mobile genetic elements, which facilitate horizontal transfer between bacterial species and accelerate the spread of resistance within clinical, animal and environmental settings (Pitout & Laupland, 2008).

Beyond healthcare facilities, Enterobacteriaceae are widely distributed in environmental reservoirs such as wastewater, rivers, soils, and aquaculture systems. Environmental contamination with antimicrobial residues, untreated waste and animal manure contributes significantly to the persistence and dissemination of resistant bacteria in aquatic ecosystems (Wellington *et al.*, 2013; Berendonk *et al.*, 2015). Fish ponds are particularly important in antimicrobial resistance surveillance because they often receive contaminants from agricultural runoff, domestic waste, and animal activities, thereby serving as reservoirs for resistant microorganisms. In addition, aquaculture environments may facilitate the exchange of resistance genes among bacterial populations due to continuous exposure to organic matter and antimicrobial selective pressure. Surveillance of fish pond water therefore provides valuable information on the environmental circulation and dissemination of resistant Enterobacteriaceae within local communities (Cabello, 2006; Shah *et al.*, 2014; Done *et al.*, 2015).

Companion animals, especially dogs, are increasingly recognized as important reservoirs of antimicrobial-resistant bacteria because of their close interaction with humans and frequent exposure to antimicrobial agents (Pomba *et al.*, 2017; Guardabassi, Schwarz, & Lloyd, 2004). Dogs may acquire resistant bacteria through contaminated food, environmental exposure, or antimicrobial treatment and subsequently transmit these organisms to humans through direct or indirect contact (Pomba *et al.*, 2017). In many Nigerian communities, local dogs are often allowed to roam freely, scavenge refuse dumps and interact extensively with contaminated environments, increasing their potential role in the spread of resistant bacteria. Monitoring antimicrobial resistance among local dogs is therefore essential for understanding the zoonotic and environmental dimensions of AMR transmission.

Despite growing global concern regarding AMR, there is limited information on the occurrence and resistance patterns of Enterobacteriaceae circulating within animal and aquatic environments in many low- and middle-income countries, including Nigeria. In Ado Ekiti, data on antimicrobial-resistant Enterobacteriaceae from local dogs and fish pond environments remain scarce. Considering the close interconnection between humans, animals, and the environment, integrated surveillance across these sectors is critical within the One Health framework. This study therefore investigated the occurrence and antimicrobial susceptibility patterns of Enterobacteriaceae isolated from Companion dogs and fish pond water in Ado Ekiti, Nigeria, in order to provide baseline data for AMR surveillance and public health intervention strategies.

Materials and Methods

Study Area and Design

This cross-sectional study was conducted in Ado-Ekiti, Ekiti State, Southwestern Nigeria. The study investigated the occurrence and antimicrobial resistance patterns of Enterobacteriaceae isolated from companion dogs and fish pond water.

Sample Collection and Processing

Non-duplicate Rectal swab specimens were aseptically collected from apparently healthy companion dogs using sterile cotton-tipped swabs, while pond water samples were obtained from selected fish ponds within the same community in Ado Ekiti, Nigeria. A total of 50 rectal swab samples and 25 pond water samples were collected during the study period. Approximately 100 mL of pond water was collected into sterile screw-capped bottles. All samples were labeled appropriately and transported immediately to the Microbiology Laboratory, Ekiti State University, Ado Ekiti, for bacteriological analysis. Rectal swabs were directly inoculated onto MacConkey agar and Eosin Methylene Blue (EMB) agar (Oxoid, UK), while pond water samples were serially diluted in sterile normal saline and cultured on the same media using the spread plate method. Plates were incubated aerobically at 37 °C for 18–24 h for selective isolation of Enterobacteriaceae. Distinct lactose-fermenting colonies were selected based on colonial morphology and repeatedly subculture on nutrient agar to obtain pure isolates, which were subsequently preserved on nutrient agar slants at 4 °C for further analysis (Chesbrough, 2006)

Microbiological Identification of Isolates

Bacterial isolates were identified using standard microbiological and biochemical methods. Preliminary identification was based on colonial morphology on MacConkey agar and Eosin Methylene Blue (EMB) agar, followed by Gram staining for confirmation of Gram-negative bacilli. Biochemical characterization was performed using conventional tests including catalase, oxidase, indole, methyl red (MR), Voges–Proskauer (VP), citrate utilization, urease, motility and Triple Sugar Iron (TSI) agar reactions, as commonly applied for differentiation of Enterobacteriaceae, particularly *Escherichia coli* and *Klebsiella pneumoniae* (Forbes *et al.*, 2017; Tille, 2022). *Escherichia coli* was identified based on indole and methyl red positivity, citrate and Voges–Proskauer negativity, motility, and acid and gas production on TSI agar while *Klebsiella pneumoniae* was identified by non-motility, citrate and Voges–Proskauer positivity, urease activity, and mucoid lactose-fermenting colonies on differential media, with an acid/acid reaction and gas production on TSI agar (Chesbrough, 2006; Forbes *et al.*, 2017; Tille, 2022).

Gram Staining

Gram staining was performed using the standard differential staining technique. A thin smear of bacterial culture was prepared on a clean glass slide and heat-fixed. The smear was stained with crystal violet for 1 minute, followed by iodine solution as a

mordant to form a crystal violet–iodine complex. Decolorization was achieved using 70% ethanol, after which the slide was counterstained with safranin. Gram-negative bacteria appeared pink/red, while Gram-positive organisms retained the purple stain (Chesbrough, 2006).

Biochemical Characterization

Indole test

The indole test was used to detect the ability of organisms to hydrolyze tryptophan to indole. Bacterial isolates from 18–24-hour cultures were inoculated into tryptone broth and incubated at 37°C for 48 hours. After incubation, Kovac's reagent was added. A red ring at the reagent layer indicated a positive result, while no color change indicated a negative reaction (Chesbrough, 2006)

Citrate Utilization Test

Simon's citrate agar slants were inoculated by streaking and incubated at 37°C for up to 7 days. Growth with a blue coloration of the medium indicated a positive result, while no growth or color change indicated a negative reaction (Chesbrough, 2006).

Urease Test

Urease production was determined by inoculating Christensen's urea agar slants and incubating at 37°C for 24–48 hours. A positive test was indicated by a pink coloration due to ammonia production, while no color change indicated a negative result (Chesbrough, 2006).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar in accordance with Clinical and Laboratory Standards Institute guidelines (CLSI 2023) State the version of this document you used. A standardized bacterial suspension equivalent to 0.5 McFarland standard was prepared from pure colonies. The inoculum was uniformly spread over Mueller Hinton agar plates using a sterile swab to obtain a confluent lawn culture. Antibiotic discs were aseptically applied to the agar surface and gently pressed to ensure full contact. The antibiotics tested include ofloxacin (OFX, 5 µg), aztreonam (ZEM, 30 µg), nalidixic acid (NA, 30 µg), ceftriaxone (CRO, 30 µg), cefuroxime (CXM, 30 µg), amoxicillin (ACX, 25 µg), cefotaxime (CTX, 30 µg), imipenem (IMP, 10 µg), amoxicillin–clavulanate (AUG, 30 µg), gentamicin (GN, 10 µg), levofloxacin (LEV, 5 µg), and nitrofurantoin (NF, 300 µg). Plates were incubated at 37°C for 18–24 hours. Zones of inhibition were measured in millimeters using a calibrated ruler and interpreted according to CLSI 2023 breakpoints.

Preliminary phenotypic carbapenem susceptibility screening

Preliminary phenotypic carbapenem susceptibility screening was conducted using imipenem and meropenem antibiotic discs. Isolates demonstrating reduced susceptibility were interpreted according to CLSI 2023 guidelines.

Quality Control

Quality control was ensured using standard reference strains, including *Escherichia coli* ATCC 25922, for validation of culture media and antimicrobial susceptibility testing procedures, in accordance with CLSI recommendations

Results

Distribution of dog samples according to breed and sex

Rectal swab samples were collected from local dogs and German Shepherd dogs. Among local dogs sampled (n = 35), 19 (54.3%) were males while 16 (45.7%) were females. Among German Shepherd dogs (n = 15), 10 (66.7%) were males and 5 (33.3%) were females.

Table 1 Distribution of dog samples according to breed and sex

| Dog breed | Sex | Frequency | Percentage (%) |
|--------------------------|--------|-----------|----------------|
| Local dogs (n = 35) | Male | 19 | 54.3 |
| | Female | 16 | 45.7 |
| German Shepherd (n = 15) | Male | 10 | 66.7 |
| | Female | 5 | 33.3 |

Identification and Characterization of Isolates

Bacterial isolates were identified based on morphological and biochemical characteristics in accordance with standard microbiological procedures. Colony morphology on MacConkey and Eosin Methylene Blue (EMB) agar revealed two predominant phenotypes. Colonies presumptively identified as *Escherichia coli* were characterized by green metallic sheen on EMB agar, motility, and non-mucoid appearance. In contrast, *Klebsiella pneumoniae* isolates produced large, mucoid, pink colonies, were non-motile, and lacked pigmentation (Table 3.2). Biochemical characterization further confirmed the identity of the isolates. *E. coli* isolates were indole-positive, citrate-negative, urease-negative and oxidase-negative, whereas *K. pneumoniae* isolates were indole-negative, citrate-positive, urease-positive and oxidase-negative (Table 3.3).

Tables 2: Morphological characteristics of Enterobacteriaceae isolates recovered from companion dogs and fish pond water

| Number of Isolates (n =37) | Gram Reaction | Colony Size | Colony Colour | Odour | Colony Edge | Elevation | Pigmentation | Motility | Presumptive Organism |
|----------------------------|---------------|-------------|----------------------|-----------|-------------|-----------|--------------|------------|-------------------------|
| 31 | GNB | 1.5 mm | Pink | Odourless | Circular | Raised | None | Non-motile | <i>Klebsiella</i> spp. |
| 6 | GNB | 1.0 mm | Green metallic sheen | Odourless | Opaque | Raised | None | Motile | <i>Escherichia coli</i> |

*Gram-negative bacilli (GNB)

Table 3: Biochemical Characteristics of *E. coli* and *K. pneumoniae* from companion Dog and Fish Pond samples

| Samples | Grams rxn | oxidase | citrate | indole | urease | catalase | motility | MR | VP | TSI | reaction in EMB agar | organisms |
|-----------|-----------|---------|---------|--------|--------|----------|----------|----|----|------------------------------|----------------------|-----------------------------|
| Dog | GNB | - | - | + | - | + | + | + | - | A/A,gas, no H ₂ S | green metallic sheen | <i>E.coli</i> |
| | GNB | - | + | - | + | + | - | - | + | A/A,gas, no H ₂ S | pink mucoid | <i>K. pneumoniae</i> |
| Fish pond | GNB | - | - | + | - | + | + | + | - | A/A,gas, no H ₂ S | green metallic sheen | <i>E. coli</i> |
| | GNB | - | + | - | + | + | - | - | + | A/A,gas, no H ₂ S | pink mucoid | <i>K. pneumoniae</i> |

Keys: GNB - Gram negative bacilli, +ve - Positive; -ve - Negative, MR=Methyl Red, VP-Voges-proskaur, TSI-Trippl Sugar Ion Test.

Prevalence of Enterobacteriaceae isolates

A total of 37 Enterobacteriaceae isolates were recovered from dog rectal swabs and fish pond water samples. Overall, *Klebsiella pneumoniae* was the predominant isolate, accounting for 31/37 (83.8%) of the total isolates, while *Escherichia coli* constituted 6/37 (16.2%). Among isolates recovered from dog samples (n = 21), *K. pneumoniae* accounted for 16/21 (76.2%), whereas *E. coli* accounted for 5/21 (23.8%). Similarly, fish pond water samples (n = 16) yielded predominantly *K. pneumoniae* isolates, representing 15/16 (93.8%), while *E. coli* accounted for 1/16 (6.2%).

TABLE 4: Prevalence of Enterobacteriaceae isolated from dog rectal swabs and fish pond water

| Sample source | Organism | Frequency | Percentage (%) |
|--------------------------|------------------------------|-----------|----------------|
| Dog samples (n = 21) | <i>Escherichia coli</i> | 5 | 23.8 |
| | <i>Klebsiella pneumoniae</i> | 16 | 76.2 |
| Fish pond water (n = 16) | <i>Escherichia coli</i> | 1 | 6.2 |
| | <i>Klebsiella pneumoniae</i> | 15 | 93.8 |
| Overall (n = 37) | <i>Escherichia coli</i> | 6 | 16.2 |
| | <i>Klebsiella pneumoniae</i> | 31 | 83.8 |

Antimicrobial Susceptibility Patterns of Isolates from Dogs

The antimicrobial susceptibility profiles of isolates obtained from dog samples are presented in figure 1. *K. pneumoniae* exhibited high levels of resistance to multiple antibiotics, including amoxicillin–clavulanate (87.5%), nitrofurantoin (81.3%) and cefuroxime (68.8%). In contrast, *E. coli* isolates showed comparatively lower resistance, although resistance to selected antibiotics was still observed. Overall, isolates from dogs demonstrated multidrug resistance (MDR) with resistance spanning multiple antibiotic classes.

Antimicrobial Susceptibility Patterns of Isolates from Fish Pond Water Samples

The antimicrobial resistance profiles of isolates from fish pond water are summarized in Figure 2. *Klebsiella pneumoniae* isolates recovered from fish pond water samples demonstrated high resistance to gentamicin (87.5%), levofloxacin (87.5%), amoxicillin–clavulanate (81.3%), and nalidixic acid (75.0%). *E. coli* isolates showed moderate resistance, with relatively similar resistance patterns across several antibiotics. Similar to dog isolates, fish pond isolates demonstrated multidrug resistance, indicating widespread resistance across major antibiotic classes.

Preliminary Phenotypic Carbapenem Susceptibility Profiles

Preliminary carbapenem susceptibility testing demonstrated resistance to imipenem among 7/28 (25.0%) *Klebsiella pneumoniae* isolates and resistance to meropenem among 2/28 (7.1%) isolates. *Escherichia coli* isolates remained susceptible to both carbapenems as presented in (Table 3.5).

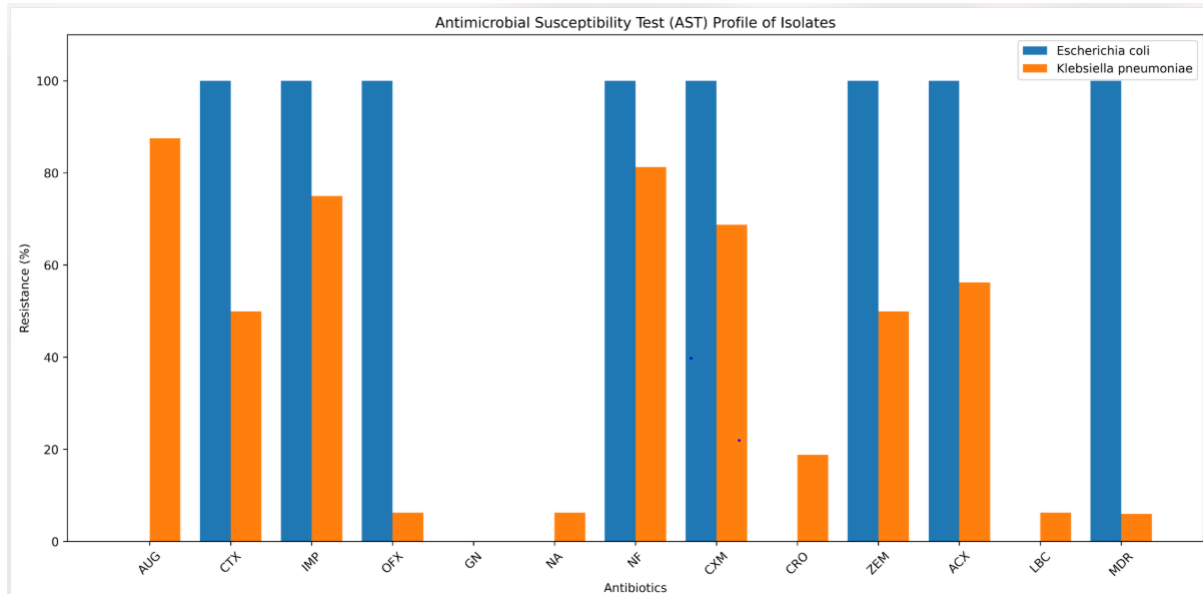


Figure 1: Overall Prevalence Antibiotic Resistance of Bacterial Isolates from dog samples

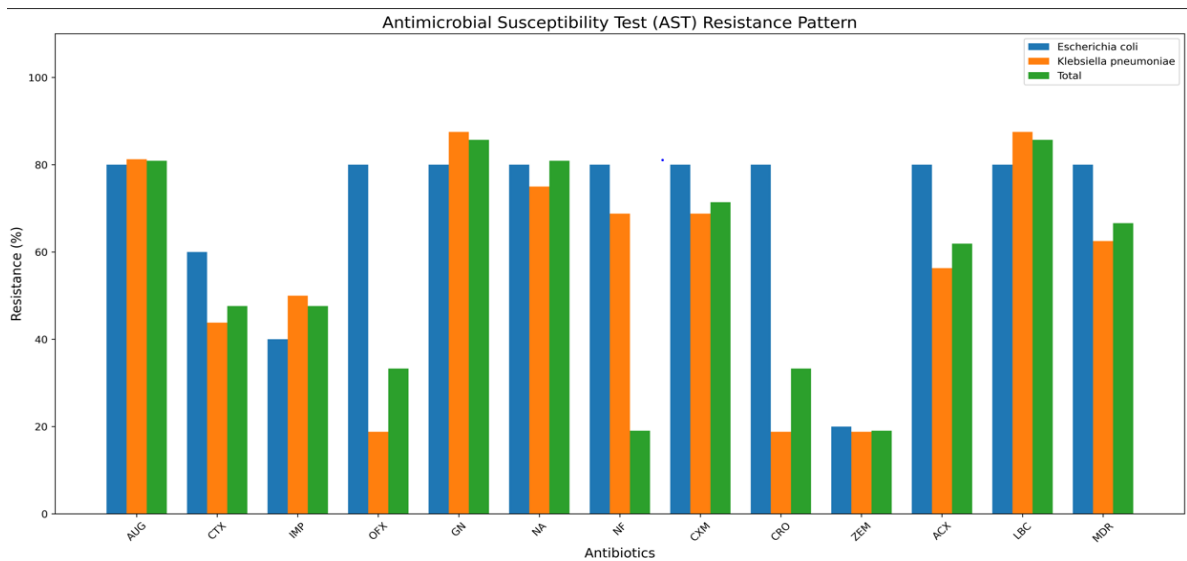


Figure 2: Overall Prevalence Antibiotic Resistance of Bacterial Isolates from Fish samples

Keys: *n*- number of isolates, amoxicillin-clavulanate, CTX-ceftriaxone; IMP-Imipenem OFX- Ofloxacin; GN- Gentamycin; NA - Nalidixic Acid; NF -Nitrofurantoin; CXM- Cefuroxime; CRO- Ceftriaxone; ZEM – Aztreonam; ACX -Amoxycillin; LBC - Levofloxacin. (CLSI 2023)

R- Resistance; I- Intermediate; S- Susceptibility, MDR- Multi-drug Resistance

Table 5 Preliminary phenotypic carbapenem susceptibility profiles of Enterobacteriaceae isolates

| Antibiotic | <i>Escherichia coli</i> (n = 5) | <i>Klebsiella pneumoniae</i> (n = 28) | Overall /Percentage |
|------------|---------------------------------|---------------------------------------|---------------------|
| Imipenem | 0 (0%) | 7 (25.0%) | 7 (21.2%) |
| Meropenem | 0 (0%) | 2 (7.1%) | 2 (6.1%) |

Discussion

The increasing emergence and dissemination of multidrug-resistant (MDR) Enterobacteriaceae constitute a major global public health challenge due to their association with severe infections, increased healthcare burden, prolonged hospitalization, therapeutic failure, and elevated mortality rates (World Health Organization, 2024; Tamma *et al.*, 2024). In the present study, *Escherichia coli* and *Klebsiella pneumoniae* were recovered from dog rectal swabs and fish pond water samples collected in Ado-Ekiti, Nigeria, indicating the circulation of resistant Enterobacteriaceae within both animal and environmental reservoirs. The recovery of these organisms from apparently healthy dogs and aquatic environments further emphasizes the epidemiological significance of companion animals and environmental water systems in the maintenance and dissemination of antimicrobial resistance (AMR). This observation is consistent with the One Health concept, which recognizes the interconnected transmission dynamics of resistant bacteria among humans, animals, and environmental compartments (Effah *et al.*, 2020; WHO, 2024).

The predominance of *K. pneumoniae* among the isolates obtained in this study corroborates previous reports describing the organism as an ecologically versatile opportunistic pathogen with remarkable adaptability to diverse environmental conditions (Wyres and Holt, 2023). The ability of *K. pneumoniae* to persist in aquatic systems, animal hosts, sewage-contaminated environments, and hospital settings has been attributed to its extensive accessory genome, polysaccharide capsule production, efficient biofilm-forming capacity, and acquisition of mobile genetic elements associated with antimicrobial resistance determinants (Navon-Venezia *et al.*, 2021; Wyres and Holt, 2023). The significantly higher prevalence of *K. pneumoniae* in fish pond water observed in this study may therefore reflect environmental contamination arising from agricultural runoff, untreated sewage discharge, poor sanitary infrastructure, and indiscriminate disposal of domestic and livestock waste into surrounding water bodies. Similar studies have identified aquatic ecosystems as important environmental reservoirs facilitating the persistence and dissemination of resistant Enterobacterales (Larsson and Flach, 2022; Adeolu *et al.*, 2024).

The antimicrobial susceptibility profiles obtained in this study demonstrated substantial resistance to multiple commonly used antibiotics among isolates recovered from both dogs and fish pond water. Notably, *K. pneumoniae* isolates from dogs exhibited high resistance to amoxicillin–clavulanate, nitrofurantoin, and cefuroxime, whereas isolates from fish pond water demonstrated elevated resistance to gentamicin, levofloxacin, nalidixic acid, and amoxicillin–clavulanate. These findings indicate extensive multidrug resistance across different antimicrobial classes, including β -lactams, aminoglycosides, quinolones, and nitrofurans. The high resistance observed may be associated with selective pressure resulting from indiscriminate antimicrobial use, empirical antibiotic therapy, over-the-counter accessibility of antimicrobial agents, self-medication practices, and poor implementation of antimicrobial stewardship programs in many developing countries (Onuoha *et al.*, 2022). Furthermore, the widespread use of antibiotics in livestock production and aquaculture may contribute significantly to environmental antibiotic contamination and subsequent selection of resistant bacterial populations (Food and Agriculture Organization, 2023).

The occurrence of MDR Enterobacteriaceae in fish pond water further reinforces the critical role of aquatic ecosystems in the persistence and environmental dissemination of antimicrobial resistance. Aquatic environments frequently receive antibiotic residues, resistant bacteria, heavy metals, and other pollutants from municipal wastewater, hospital effluents, agricultural runoff, and aquaculture activities, thereby creating ecological conditions that favor co-selection and maintenance of resistance determinants (Larsson and Flach, 2022). Continuous exposure of environmental bacteria to sub-inhibitory concentrations of antibiotics may facilitate horizontal gene transfer events and enrichment of resistance genes within microbial communities (Tamma *et al.*, 2024). Consequently, fish ponds and other aquatic environments may function as ecological hotspots for the emergence and amplification of antimicrobial-resistant pathogens of public health importance. Similar observations have been documented in environmental surveillance studies conducted in sub-Saharan Africa, where aquatic systems were identified as reservoirs of clinically relevant resistant Enterobacterales (Adeolu *et al.*, 2024).

Although phenotypic susceptibility testing in this study showed lower resistance rates to carbapenems compared with other antimicrobial classes, the detection of reduced susceptibility among some *K. pneumoniae* isolates remains noteworthy because carbapenems are critically important antibiotics reserved for severe Gram-negative bacterial infections (Centers for Disease Control and Prevention, 2024). However, since only phenotypic methods were employed in the present study,

definitive characterization of the underlying resistance mechanisms could not be established. Further molecular investigations are therefore required to determine the presence of specific resistance determinants and to better understand the genetic basis of reduced carbapenem susceptibility among the isolates.

The findings of this study strongly support the growing evidence for zoonotic and environmental transmission pathways involved in the epidemiology of antimicrobial resistance. Companion animals, particularly dogs, may act as asymptomatic carriers of resistant Enterobacteriaceae due to their close and prolonged interaction with humans. Several studies have demonstrated genomic similarities between resistant Enterobacterales isolated from companion animals and those recovered from human clinical infections, suggesting possible bidirectional transmission of resistant strains and resistance genes (Effah *et al.*, 2020; Pomba *et al.*, 2023). Likewise, contaminated aquatic environments may facilitate dissemination of resistant organisms through direct environmental exposure, irrigation systems, aquaculture products, and contaminated food chains. These transmission pathways underscore the importance of integrated One Health surveillance systems incorporating veterinary, environmental, and human health sectors.

Although comparatively lower resistance rates were observed against certain antibiotics, these findings should be interpreted cautiously because antimicrobial resistance patterns remain highly dynamic and continuously evolving under selective environmental pressures. Reduced resistance to specific antimicrobial agents may reflect limited usage within the study area or reduced exposure of bacterial populations to those antibiotics. Nevertheless, the coexistence of susceptible and highly resistant phenotypes within the same ecological environment highlights the complexity of antimicrobial resistance evolution and dissemination. Strengthening antimicrobial stewardship policies, restricting indiscriminate antibiotic use, improving environmental sanitation, implementing wastewater treatment strategies, and promoting responsible antimicrobial practices in veterinary and aquaculture settings remain essential interventions for mitigating the spread of antimicrobial resistance (WHO, 2024; FAO, 2023).

Overall, the public health implications of the present findings are substantial. The detection of multidrug-resistant Enterobacteriaceae in dog rectal swabs and fish pond water suggests the existence of environmental and zoonotic reservoirs capable of facilitating transmission of resistant bacteria and resistance genes to humans. Such transmission may occur through direct contact with companion animals, exposure to contaminated aquatic environments, or consumption of contaminated aquatic

products. Consequently, the findings highlight the urgent need for continuous AMR surveillance, molecular characterization of resistance determinants, effective antimicrobial stewardship programs, environmental monitoring, and implementation of coordinated One Health interventions aimed at reducing the emergence and dissemination of antimicrobial resistance in Nigeria and globally.

Conclusion

This study confirmed the presence of multidrug-resistant *Escherichia coli* and *Klebsiella pneumoniae* in dog rectal swabs and fish pond water in Ado-Ekiti, Nigeria, indicating that companion animals and aquatic environments act as important reservoirs of antimicrobial-resistant Enterobacteriaceae. The predominance of *K. pneumoniae* and the high resistance to commonly used antibiotics highlight the ongoing spread of resistant bacteria in animal and environmental sources. These findings emphasize the public health risk of antimicrobial resistance transmission within a One Health framework. Further molecular studies are recommended to identify resistance genes and mechanisms. Strengthening antimicrobial stewardship, environmental hygiene, and coordinated One Health surveillance is essential to curb the spread of antimicrobial resistance.

Acknowledgements

The authors acknowledge the Department of Medical Laboratory Science and the Department of Microbiology, Ekiti State University, Ado-Ekiti, Nigeria, for providing laboratory facilities and technical support for this study.

Funding

The authors received no external funding for this study.

Conflict Of Interest

The authors declare no conflict of interest.

.

Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

References

- Adeolu, M., Akinyemi, K. O., & Oladipo, E. K. (2024). Antimicrobial resistance in aquaculture environments in Southwestern Nigeria. *Environmental Microbiology Reports*, 16(2), 145–156. <https://doi.org/10.1111/1758-2229.13245>
- Alhassan, A. J., Adu, F., & Bawa, M. (2021). Occurrence of multidrug-resistant *Klebsiella pneumoniae* in environmental and animal sources. *African Journal of Clinical and Experimental Microbiology*, 22(4), 456–464. <https://doi.org/10.4314/ajcem.v22i4.8>
- Berendonk, T. U., Manaia, C. M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Bürgmann, H., Sørum, H., Norström, M., Pons, M. N., Kreuzinger, N., Huovinen, P., Stefani, S., Schwartz, T., Kisand, V., Baquero, F., & Martinez, J. L. (2015). Tackling antibiotic resistance: The environmental framework. *Nature Reviews Microbiology*, 13(5), 310–317. <https://doi.org/10.1038/nrmicro3439>
- Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. *Environmental Microbiology*, 8(7), 1137–1144. <https://doi.org/10.1111/j.1462-2920.2006.01054.x>
- Cappuccino, J. G., & Sherman, N. (2014). *Microbiology: A laboratory manual* (10th ed.). Pearson Education.
- Centers for Disease Control and Prevention. (2024). *Carbapenem-resistant Enterobacteriales (CRE)*. U.S. Department of Health and Human Services. <https://www.cdc.gov/cre/index.html>
- Clinical and Laboratory Standards Institute. (2023). *Performance standards for antimicrobial susceptibility testing* (33rd ed.). CLSI.
- Done, H. Y., Venkatesan, A. K., & Halden, R. U. (2015). Does the recent growth of aquaculture create antibiotic resistance threats different from those associated with land animal production in agriculture? *The AAPS Journal*, 17(3), 513–524. <https://doi.org/10.1208/s12248-015-9722-z>
- Effah, C. Y., Sakyi, S. A., Aboagye, S. Y., & Amuah, E. E. Y. (2020). Multidrug-resistant *Escherichia coli* and *Klebsiella pneumoniae* in companion animals and humans: Public health implications. *Frontiers in Microbiology*, 11, 587015. <https://doi.org/10.3389/fmicb.2020.587015>
- Food and Agriculture Organization. (2023). *The role of aquaculture in antimicrobial resistance*. FAO Publications. <https://www.fao.org>
- Forbes, B. A., Sahm, D. F., & Weissfeld, A. S. (2017). *Bailey & Scott's diagnostic microbiology* (14th ed.). Elsevier.

- Guardabassi, L., Schwarz, S., & Lloyd, D. H. (2004). Pet animals as reservoirs of antimicrobial-resistant bacteria. *Journal of Antimicrobial Chemotherapy*, *54*(2), 321–332. <https://doi.org/10.1093/jac/dkh332>
- Larsson, D. G. J., & Flach, C. F. (2022). Antibiotic resistance in the environment. *Nature Reviews Microbiology*, *20*(5), 257–269. <https://doi.org/10.1038/s41579-021-00649-x>
- Murray, C. J. L., Ikuta, K. S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A., Han, C., Bisignano, C., Rao, P., Wool, E., Johnson, S. C., Browne, A. J., Chipeta, M. G., Fell, F., Hackett, S., Haines-Woodhouse, G., Kashef Hamadani, B. H., Kumaran, E. A. P., McManigal, B., ... Naghavi, M. (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *The Lancet*, *399*(10325), 629–655. [https://doi.org/10.1016/S0140-6736\(21\)02724-0](https://doi.org/10.1016/S0140-6736(21)02724-0)
- Navon-Venezia, S., Kondratyeva, K., & Carattoli, A. (2021). *Klebsiella pneumoniae*: A major worldwide source and shuttle for antibiotic resistance. *FEMS Microbiology Reviews*, *45*(4), fuab010. <https://doi.org/10.1093/femsre/fuab010>
- Onuoha, S. C., Fatokun, K., & Adekanmbi, A. O. (2022). Antimicrobial susceptibility patterns of Enterobacteriaceae from animal and environmental sources in Nigeria. *Veterinary World*, *15*(9), 2213–2221. <https://doi.org/10.14202/vetworld.2022.2213-2221>
- Pitout, J. D. D., & Laupland, K. B. (2008). Extended-spectrum β -lactamase-producing Enterobacteriaceae: An emerging public-health concern. *The Lancet Infectious Diseases*, *8*(3), 159–166. [https://doi.org/10.1016/S1473-3099\(08\)70041-0](https://doi.org/10.1016/S1473-3099(08)70041-0)
- Pomba, C., Rantala, M., Greko, C., Baptiste, K. E., Catry, B., van Duijkeren, E., Mateus, A., Moreno, M. A., Pyörälä, S., Ružauskas, M., Sanders, P., Teale, C., Threlfall, E. J., Kunsagi, Z., Torren-Edo, J., Jukes, H., & Törneke, K. (2017). Public health risk of antimicrobial resistance transfer from companion animals. *Journal of Antimicrobial Chemotherapy*, *72*(4), 957–968. <https://doi.org/10.1093/jac/dkw481>
- Pomba, C., Rantala, M., Greko, C., Baptiste, K. E., Catry, B., van Duijkeren, E., Mateus, A., Moreno, M. A., Pyörälä, S., Ružauskas, M., Sanders, P., Teale, C., & Threlfall, E. J. (2023). Public health risk of antimicrobial resistance transfer from companion animals. *Journal of Antimicrobial Chemotherapy*, *78*(3), 611–620. <https://doi.org/10.1093/jac/dkad012>
- Shah, S. Q. A., Cabello, F. C., L'Abée-Lund, T. M., Tomova, A., Godfrey, H. P., Buschmann, A. H., & Sørum, H. (2014). Antimicrobial resistance and antimicrobial resistance genes in marine bacteria from salmon aquaculture and non-aquaculture sites. *Environmental Microbiology*, *16*(5), 1310–1320. <https://doi.org/10.1111/1462-2920.12421>

- Tamma, P. D., Aitken, S. L., Bonomo, R. A., Mathers, A. J., van Duin, D., & Clancy, C. J. (2024). Infectious Diseases Society of America guidance on the treatment of antimicrobial-resistant Gram-negative infections. *Clinical Infectious Diseases*, 78(4), 889–898. <https://doi.org/10.1093/cid/ciad428>
- Tille, P. (2022). *Bailey & Scott's diagnostic microbiology* (15th ed.). Elsevier.
- van Duin, D., & Doi, Y. (2017). The global epidemiology of carbapenemase-producing Enterobacteriaceae. *Virulence*, 8(4), 460–469. <https://doi.org/10.1080/21505594.2016.1222343>
- Wellington, E. M., Boxall, A. B., Cross, P., Feil, E. J., Gaze, W. H., Hawkey, P. M., Johnson-Rollings, A. S., Jones, D. L., Lee, N. M., Otten, W., Thomas, C. M., & Williams, A. P. (2013). The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. *The Lancet Infectious Diseases*, 13(2), 155–165. [https://doi.org/10.1016/S1473-3099\(12\)70317-1](https://doi.org/10.1016/S1473-3099(12)70317-1)
- World Health Organization. (2024). *Global antimicrobial resistance and use surveillance system (GLASS) report 2024*. World Health Organization. <https://www.who.int/publications/i/item/9789240090996>
- Wyres, K. L., & Holt, K. E. (2023). Population genomics of *Klebsiella pneumoniae*. *Nature Reviews Microbiology*, 21(5), 324–337. <https://doi.org/10.1038/s41579-022-00801-7>

