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# Antibiotic sensitivity of bacterial isolates from farmed fish in Uasin Gishu County, Kenya

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**The study was conducted with the aim of investigating the antibiotic sensitivity of bacterial isolates from farmed fish to commonly used antibiotics in Uasin Gishu County, Kenya, a region experiencing rapid aquaculture growth. Despite no direct antibiotic use in local fish farms, concerns over antimicrobial resistance (AMR) have emerged due to indirect exposure from integrated livestock-crop systems. A longitudinal study design was used to evaluate microbial infestations. Over a 12-month period, 468 fish samples from 13 ponds across five farms were examined, yielding 154 bacterial isolates representing 12 species, including *Escherichia coli*, *Aeromonas* species, *Pseudomonas aeruginosa*, *Vibrio* species and *Flavobacterium columnare*. Antibiotic susceptibility testing was conducted using six commonly used antibiotics: amoxicillin (AMX), erythromycin (ERY), cotrimoxazole (CT), doxycycline (DO), streptomycin (STR), and ciprofloxacin (CIP). Statistical analysis using Fisher's exact test revealed significant interspecies resistance variation for AMX and ERY ($p = 0.0005$), while the other antibiotics showed consistent susceptibility across species. Principal Component Analysis (PCA) and heatmap analyses confirmed AMX and ERY as the primary drivers of resistance variation, particularly in *E. coli*, *Aeromonas hydrophila* and *Streptococcus* species, which showed resistance rates above 35 to 50%. Conversely, CIP, DO, and STR exhibited high effectiveness, with over 80% susceptibility in most isolates. The results suggest that indirect exposure through runoff, manure application, and effluent from livestock farming is likely promoting resistance. The findings of this study reveal that antibiotic use in surrounding agricultural systems, although not directly applied to fish, may be contributing to the development of resistant bacterial populations within aquaculture environments. This highlights a critical link between terrestrial and aquatic ecosystems, reinforcing the need for a One Health approach to antimicrobial stewardship. The results emphasize the importance of regular monitoring of antibiotic resistance patterns and adopting tailored antibiotic selection based on local data. Furthermore, the study underscores the necessity of improved waste management, enhanced farmer education, and strengthened biosecurity practices to curb the spread of resistant bacteria. Continued surveillance of antibiotic resistance in aquaculture is essential to ensure food safety, safeguard public health, and promote the sustainability of fish farming systems.**

**Key words:** Antibiotic resistance, aquaculture, bacterial isolates, fish farming, principal component analysis (PCA)

# INTRODUCTION

Fisheries production has undergone a transformative shift decades (Food and Agriculture Organisation (FAO), with the rapid expansion of aquaculture, one of the 2018; Pradeepkiran, 2019). It has become a vital world's fastest-growing food production sectors in recent contributor to global food security, providing an essential protein source to an increasing global human population (Allison, 2011; Edwards et al., 2019; Makori et al., 2017). With the world's human population projected to reach 10 billion by 2050, global food production must expand by up to 56% to meet demand (Allison, 2011; FAO, 2018).

Uasin Gishu County, located in Kenya's North Rift region, has seen substantial growth in aquaculture due to government initiatives promoting fish farming (Uasin Gishu Government, 2015). Despite this growth, sustainable aquaculture can be challenged by poor water quality and the prevalence of microbial and parasitic infections, which negatively impact fish health and productivity (Dabi, 2022; Ferreira et al., 2022; Zanchett and Oliveira-Filho, 2013). Additionally, antimicrobial resistance (AMR) in aquaculture has emerged as a significant concern due to the misuse of antibiotics, leading to the development of resistant bacterial strains (Dayie et al., 2024; Huavas et al., 2024).

The problem of reduced antibiotic sensitivity among bacterial isolates from farmed fish is not limited to Kenya but reflects a broader regional and global concern. Systematic evidence from Asia, based on 749 pointprevalence surveys conducted between 2000 and 2019, indicates that roughly one-third of tested antimicrobial compounds per survey had resistance rates exceeding 50% (P50 approximately 33%) in aquatic food animals (Schar et al., 2021). In Africa, a recent meta-analysis reported multidrug resistance rates of approximately 43.1% for *Escherichia coli*, 40.3% for *Salmonella* species, and 31.3% for *Staphylococcus* species, isolated from aquaculture settings (Moffo et al., 2024).

Similarly, a study in Kenya found that 86.36% of bacterial isolates from retail Nile tilapia exhibited multidrug resistance, with multiple antibiotic resistance (MAR) indices greater than 0.30 (Mumbo et al., 2023). These findings together demonstrate that antimicrobial resistance in aquaculture is widespread and a matter of both regional and global public health concern.

AMR in aquaculture has emerged as a major concern due to the excessive use of antibiotics, leading to the development of drug-resistant bacterial strains (Du et al., 2022). There is an urgent need to assess antibiotic sensitivity patterns among fish pathogens to inform responsible use of antibiotics and improve fish health management in the region. The presence of drugresistant pathogens in fish farming environments threatens both aquaculture sustainability and public health, as these pathogens may be transmitted to humans through the consumption of contaminated fish (Cabello, 2006; Souza et al., 2019). Assessing antibiotic resistance patterns in fish pathogens will inform strategies for responsible antibiotic use, thereby mitigating the risks associated with antimicrobial resistance.

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# MATERIALS AND METHODS

## Study area

The study was conducted from May 2023 to April 2024 in selected fish farms across five sub-counties in Uasin Gishu County (UG) as shown in Figure 1. Uasin Gishu County, located in the north west of the Great Rift Valley region, lies approximately 330 km northwest of Nairobi. It covers an area of 2,955 km² and characterized by a highland plateau with several perennial rivers and swampy wetlands (Basweti et al., 2009; Petrikova et al., 2024). The region experiences a temperate climate, with annual temperatures ranging between 7 to 29°C and rainfall between 624.9 mm to and 1560.4 mm per year (Njonge, 2023). Aquaculture has been expanding in Uasin Gishu in the last decade, with 1,728 operational fish ponds covering 486,000 m², producing approximately 593,000 kg of fish annually (Uasin Gishu Government, 2015).

Most farmers practicing fish farming use earthen ponds, liner ponds, and other water reservoirs. Nile tilapia (*Oreochromis niloticus*) is the predominant species farmed, followed by African catfish (*Clarias gariepinus*) (Mwangi et al., 2024).

## Study sites

Five aquaculture farms were purposively selected based on their features such as; pond type, size, location, inputs (e.g., fertilizers, manure, fish feeds), and duration of operation (minimum of one year) as indicated in Table 1. Each pond in the selected farm site was sampled monthly for 12 months to monitor water quality and fish health.

### Study design and sample size calculation

A longitudinal study design was used to evaluate the diversity and distribution of bacterial in fish cultured in the selected farms. A stratified random sampling approach was used to ensure representation across pond types, locations, and management practices. Each pond was sampled monthly for water quality assessment and fish health monitoring. A total of 13 fish ponds in five farms across four sub-counties were assessed. The sample size for fish was determined using Thrusfield's formula (Saville and Wittum, 2004) with a 95% confidence interval and 5% absolute precision.

### Fish sampling, microbial analysis and parasitological analysis

#### *Fish sampling*

Fish were collected using seine nets and transported in cool boxes to the laboratory. The condition factor (K) was determined using Froese's formula (Froese, 2006).

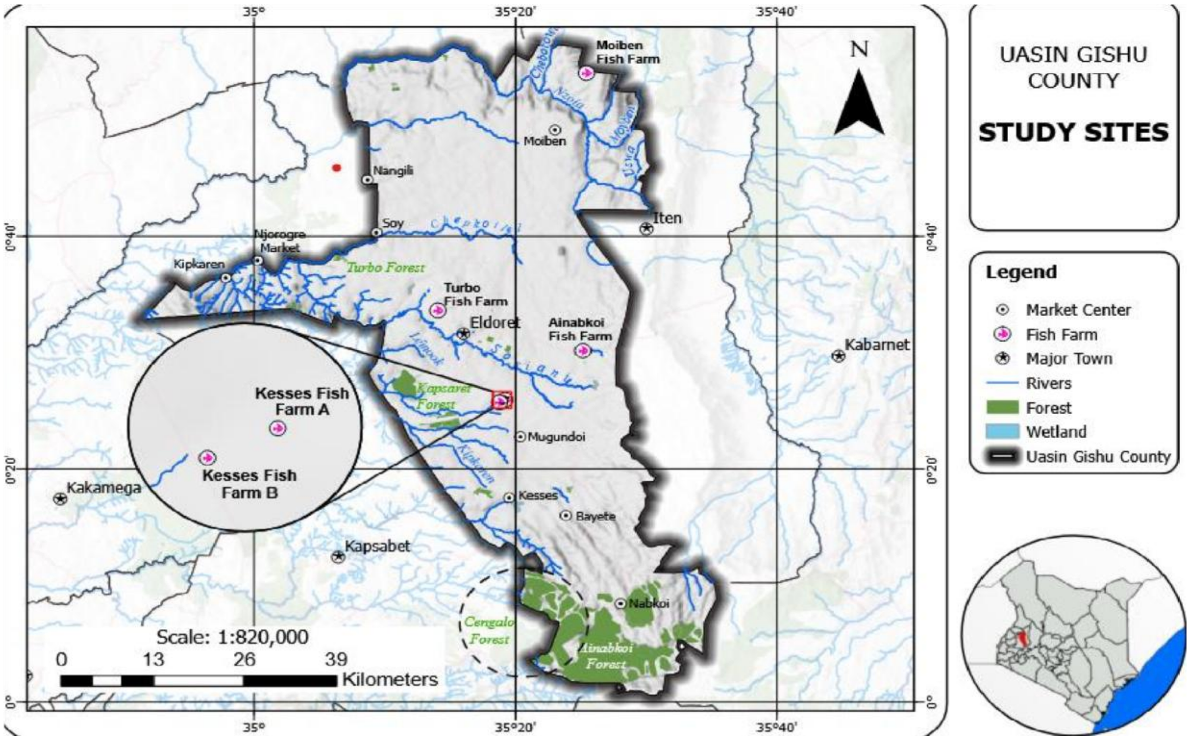

**Figure 1.** Map of Uasin Gishu showing its location in western Kenya with perennial rivers and swampy wetlands.

**Table 1.** Location and Features of the Selected Fish farms in Uasin Gishu County where the study was carried out.

| Sub-County | Farm site name | Pond | Type of ponds |
|---|---|---|---|
| Moiben | Moiben farm (Farm A) | P1 | Raised liner pond |
| | | P2 | Earthen pond |
| | | P3 | Earthen pond |
| | | P4 | Earthen pond |
| Ainapkoi | Ainapkoi farm (Farm C) | P1 | Ground liner pond |
| | | P2 | Ground liner pond |

| | | | |
|---|---|---|---|
| | | P3 | Earthen pond |
| Kesses | Kesses farm 1 (Farm K) | P1 | Raised liner pond |
| | | P2 | Raised liner pond |
| Kesses | Kesses farm 2 ( Farm S) | P1 | Earthen pond |
| | | P2 | Earthen pond |
| Turbo | Turbo farm (Farm T) | P1 | Ground liner pond |
| | | P2 | Ground liner pond |

### *Bacterial isolation and identification*

Fish tissue samples from the intestine, skin, and gills were collected aseptically and homogenized in 0.1% peptone water. Samples (0.1 mL) were inoculated onto selective media: Rimler Shotts Medium Base agar (*Aeromonas* species), Pseudomonas Base agar (*Pseudomonas* species), Thiosulfate Citrate Bile salts Sucrose (TCBS) agar (*Vibrio* species), Tryptic Soy Agar (TSA), and Brain Heart Infusion (BHI) agar. The inoculated plates were incubated at 37ºC for 24 h.

Pure isolates were characterized using morphological and biochemical tests, including Gram staining, oxidase, catalase, motility, indole, urease and citrate tests. The identification followed Bergey's manual of bacteriology (John, 1998).

### *Antimicrobial susceptibility testing*

The susceptibility of isolates was assessed using the standard disc diffusion method on Mueller-Hinton agar, following CLSI (Clinical and Laboratory Standards Institute) guidelines. Six antibiotics were selected for resistance profiling based on common usage in aquaculture and veterinary settings: Amoxicillin (AMX), Erythromycin (ERY), Cotrimoxazole (CT), Doxycycline (DO), Streptomycin (STR), and Ciprofloxacin (CIP). All discs were purchased from Oxoid Ltd.

**Table 2.** Summary of Bacterial Isolates Identified from Fish Samples in Uasin Gishu County.

| Bacterial species | Gram reaction | Colony morphology and key biochemical traits |
|---|---|---|
| Gram-positive bacteria | | |
| *Bacillus spp.* | Positive | Large, dry, irregular colonies; amoebic-like shape; hemolytic; catalasepositive, spore-forming, motile, starch hydrolysis. |
| *Streptococcus spp.* | Positive | Pinpoint, purple, non-hemolytic colonies; chains of cocci; ferments glucose and lactose. |
| *Micrococcus spp.* | Positive | Small, yellow colonies; cocci in tetrads/clusters; catalase-positive, oxidasepositive. |
| Gram-negative bacteria | | |
| *E. coli* | Negative | Pink colonies on MacConkey agar (lactose fermenter); catalase-negative, oxidase-negative, glucose fermenter (acid and gas). |
| *P. aeruginosa* | Negative | Green pigment on blood agar (pyocyanin); β-hemolysis; oxidase-positive, catalase-positive. |
| *A. hydrophila* | Negative | Off-white, circular colonies on BA; β-hemolytic; oxidase-positive, fermentative. |
| *A. caviae* | Negative | Similar to *A. hydrophila* but differentiated by oxidase and glucose fermentation patterns. |
| *A. sobria* | Negative | Similar colony morphology and hemolysis as other *Aeromonas* species; Gram-negative rod. |
| *V. harveyi* | Negative | Yellow colonies on TCBS (sucrose fermenter); halophilic; nitrate reduction. |
| *V. alginolyticus* | Negative | Yellow colonies on TCBS; sucrose fermenter; $H_2S$ production. |
| *F. columnare* | Negative | Non-hemolytic, yellow colonies on BA; oxidase-negative, starch hydrolysis. |
| *Plesiomonas spp.* | Negative | Small, non-hemolytic colonies; oxidase-positive, Gram-negative rod. |

### Statistical analysis

Fisher's exact test was employed to determine whether significant differences existed in resistance patterns among bacterial species for each antibiotic. A significance threshold of $p < 0.05$ was used. To complement the statistical analysis, Principal Component Analysis (PCA) was used to identify key patterns in antimicrobial resistance. All statistical analyses were conducted using R software 4.4.2 (Pile of Leaves) with $p \leq 0.05$ considered statistically significant.

## RESULTS

### Bacterial organisms isolated from selected fish farms

A total of 468 fish samples collected from selected fish farms in Uasin Gishu County were investigated for bacterial characterization over a one-year period, resulting in 154 bacterial colony isolates. The 154 bacterial colony isolates displayed a variety of distinct cultural and biochemical characteristics that enabled for their identification. These isolates were distributed across 12 bacterial species, including both Gram-positive and Gram-negative organisms, each exhibiting unique colony morphology and biochemical properties as shown in Table 2.

### Antibiotic sensitivity of bacterial isolates from farmed fish to commonly used antibiotics.

The results for antibiotic resistance profiles of twelve bacterial species assessed across six antibiotics: AMX, CIP, CT, DO, ERY and STR, are shown in Table 3. AMX and ERY exhibited highly significant interspecies variability in resistance. Both antibiotics yielded p-values of 0.0005, well below the conventional significance threshold of 0.05. This indicates that susceptibility to these two drugs was not uniform across the bacterial isolates, suggesting species-specific resistance dynamics. In contrast, the remaining four antibiotics; CT, DO, STR and CIP, did not show statistically significant variation in resistance patterns among the bacterial species. CT yielded a p-value of 0.3183, DO 0.3618, STR 0.7561, and CIP 0.8541. These results suggest that resistance to these antibiotics was relatively consistent across the bacterial isolates examined. Overall, the data point to significant differences in bacterial responses to AMX and ERY, while resistance p < 0.05).

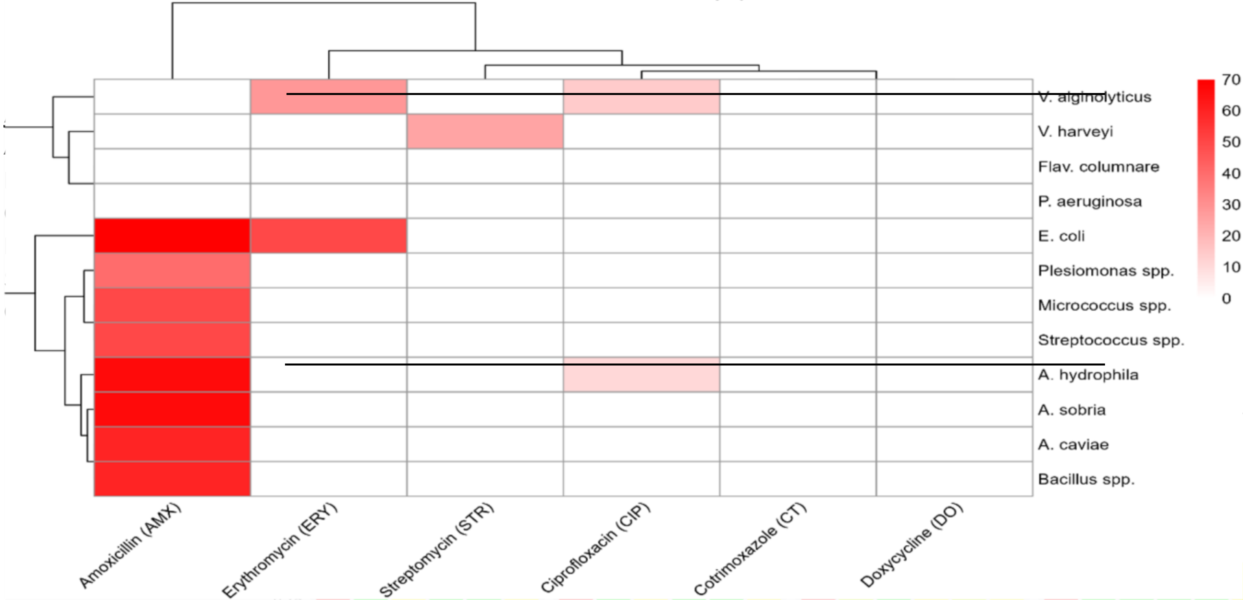

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**Figure 3.** Heatmap shc
represents the level of re

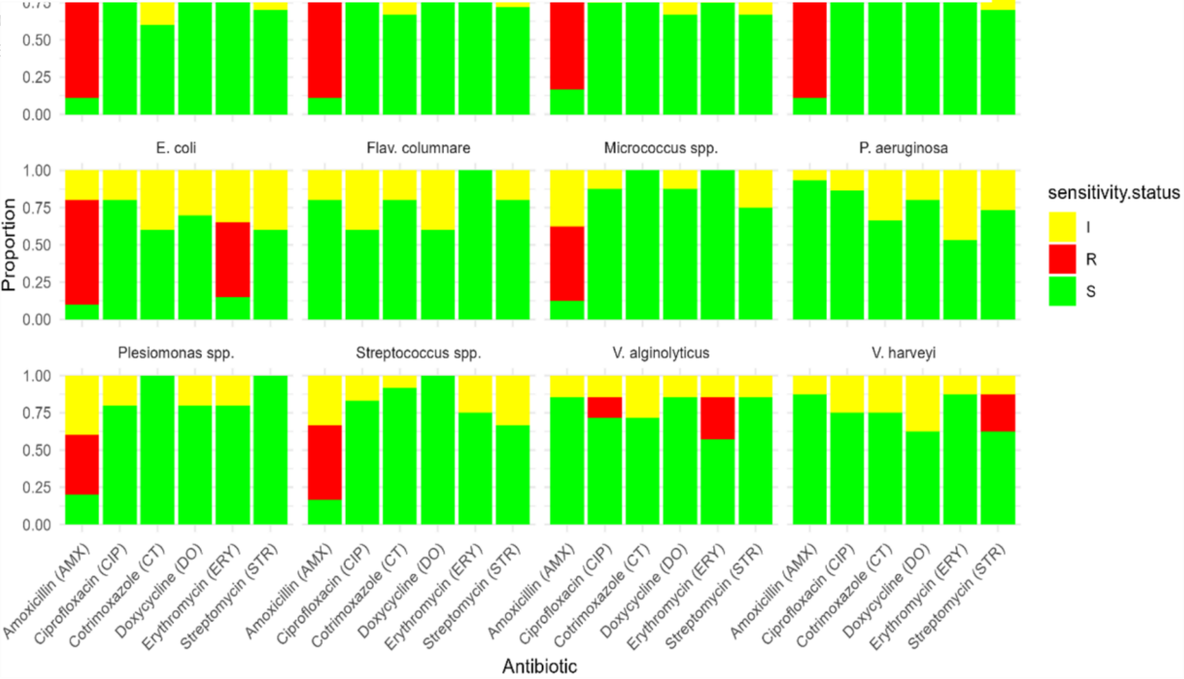

**Figure 2.** Antibiotic resistance patterns of bacterial isolates from fish farms in Uasin Gishu County.

to CT, DO, STR and CIP appeared more uniformly distributed regardless of bacterial species.

The results for antibiotic resistance patterns assessed across six antibiotics are presented in Figure 2. Overall, ciprofloxacin, doxycycline, and streptomycin exhibited the highest efficacy, with susceptibility rates exceeding 80% in most bacterial groups. In contrast, amoxicillin and erythromycin demonstrated reduced effectiveness, with resistance observed in up to 60% of isolates for certain taxa.

Within the *Aeromonas* genus, *Aeromonas caviae* and *Aeromonas sobria* showed particularly high resistance to amoxicillin, with approximately 45 to 55% of isolates classified as resistant, while *Aeromonas hydrophila* remained largely susceptible (≥85%) across the antibiotic panel. Moderate resistance to erythromycin was also observed in *A. caviae* (about 30%) and *A. sobria* (nearly 35%). In contrast, *Bacillus* species and *Micrococcus* species demonstrated strong susceptibility profiles, with ≥90% of isolates responding to all antibiotics tested, and minimal intermediate responses (typically under 10%).

*E. coli* exhibited moderate resistance to AMX and ERY, with approximately 30 to 35% of isolates resistant, though susceptibility to CIP, DO, and STR remained high (≥70%). *Flavobacterium columnare* showed nearcomplete sensitivity to all six antibiotics, with resistance levels below 5%. Similarly, *Pseudomonas aeruginosa* demonstrated consistent susceptibility across the board, with >85% of isolates classified as sensitive for each antibiotic.

Notable resistance patterns were seen in *Plesiomonas* species and *Streptococcus* species, particularly to AMX and ERY, where resistance rates reached 40 to 50%. However, these genera maintained high susceptibility (≥80%) to CIP and DO. The *Vibrio* spp. (*Vibrio alginolyticus and Vibrio harveyi*) showed greater heterogeneity in resistance patterns. *V.* harveyi, for instance, exhibited resistance in ~30% of isolates to both erythromycin and cotrimoxazole, while *V. alginolyticus* showed slightly lower resistance at around 20 to 25% for these antibiotics.

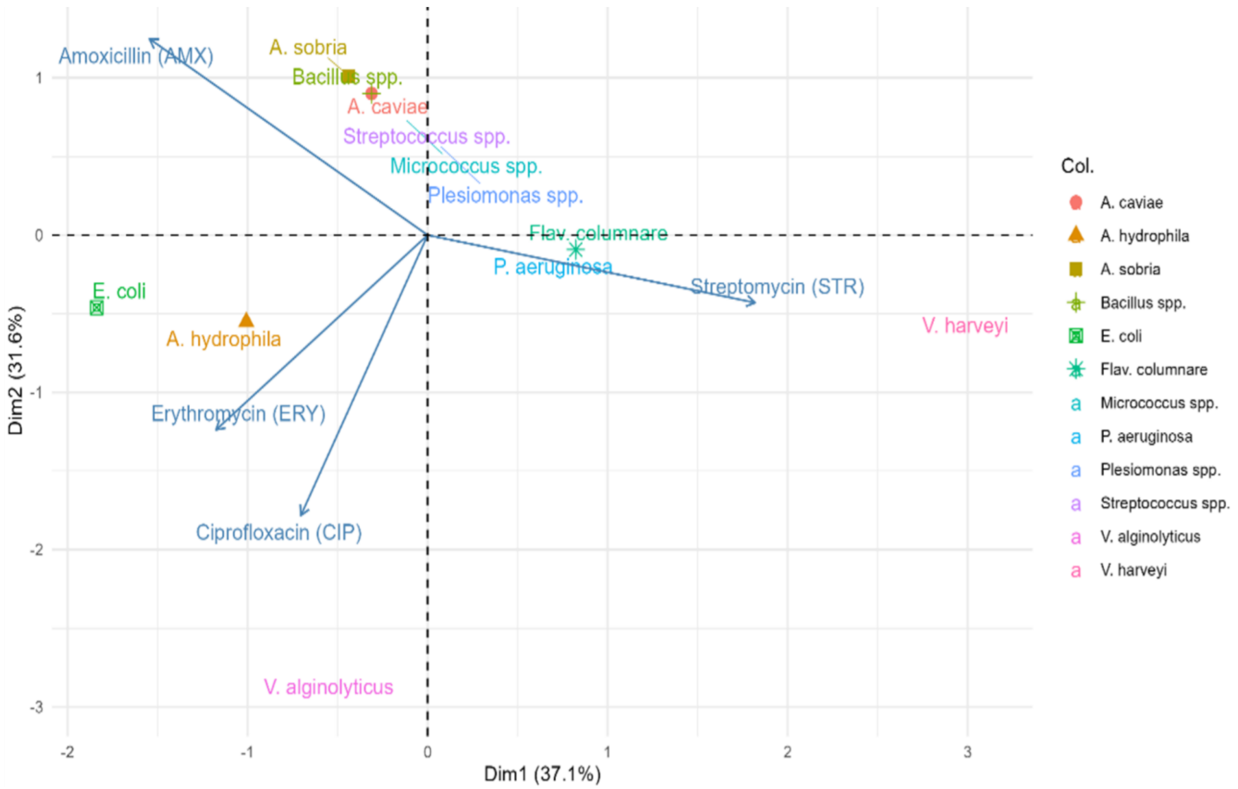

**Figure 4.** PCA biplot illustrating variation in bacterial resistance to six antibiotics. (Arrows represent antibiotics, and their directions indicate the contribution to variance along principal components).

The study revealed that CIP, DO, and STR were the most consistently effective antibiotics, with over 80% susceptibility across most bacterial species. In contrast, AMX and ERY were the least effective, with resistance rates ranging from 30% to over 50% in several taxa, particularly among *Aeromonas, Streptococcus* and *Vibrio* species. These findings highlight the critical need for targeted antibiotic selection based on species-level resistance patterns to optimize therapeutic outcomes and mitigate resistance development.

Figure 3 illustrates the antibiotic resistance profiles of bacterial isolates using a heatmap with hierarchical clustering. The clustering reveals clear patterns of elevated resistance to AMX and ERY among several isolates, particularly *E. coli*, *A. hydrophila*, and *Streptococcus spp.*, which show strong red signals indicating resistance levels above 60%. In contrast, antibiotics such as CIP, CT, and DO showed minimal resistance across all isolates, reflected by the predominance of white or pale pink shading. The dendrogram also groups species with similar resistance behaviors, highlighting the coherence of certain taxa in their antibiotic responses. These visual patterns align with statistical findings from Fisher's exact test, which confirmed significant interspecies variation only for AMX and ERY.

The PCA biplot (Figure 4) summarizes the multidimensional antibiotic resistance data by reducing it into two principal components: Dim1 (37.1% variance) and Dim2 (31.6% variance). AMX and ERY project strongly along the positive Y- axis and negative Y-axis respectively, suggesting they are key drivers of resistance variability among the isolates. *E. coli* and *A. hydrophila* appear more distant from the origin in relation to these two antibiotics, indicating species-specific resistance trends. In contrast, antibiotics like CIP, CT and DO, which are less variable according to Fisher's test, contribute minimally to dimensional separation and cluster near the origin.

The species clusterings further reinforce the heatmap and statistical analysis findings, where isolates with similar resistance responses are grouped together. For instance, *V. harveyi* and *V. alginolyticus* lies away from others, indicating distinct resistance behaviors. This PCA visualization supports the interpretation that AMX and ERY are the most discriminative agents in explaining resistance diversity across the bacterial spectrum.

## DISCUSSION

The study identified significant interspecies variability in resistance to amoxicillin and erythromycin among bacterial isolates from fish farms in Uasin Gishu County. Fisher's exact test revealed this variation to be statistically significant ($p = 0.0005$), emphasizing nonuniform resistance profiles across taxa. Resistance rates exceeded 35 to 50% in *E. coli*, *A. hydrophila*, and *Streptococcus spp*., as visualized in the heatmap (Figure 2).

This interspecies variability is likely driven by several factors, such as horizontal gene transfer (HGT), which facilitates the spread of resistance genes among environmental bacteria. This process is particularly potent in complex ecosystems like aquaculture, where microbial communities interact within shared water systems. As described by Sun et al. (2025), the persistence and mobility of antibiotic resistance genes (ARGs) in livestock and poultry settings can lead to their accumulation in the environment via effluent, manure, and runoff, eventually reaching aquatic systems through integrated farming practices.

Data from poultry and pig farms also provide compelling parallels. Gudda et al. (2024) documented antibiotic residues in 25.8% of chicken compost samples and 23.1% of pig-manure-fertilized soils, with trimethoprim concentrations exceeding safety thresholds. Resistance risk increased with flock size and antibiotic use intensity, suggesting significant environmental loading of antimicrobials even in terrestrial systems linked to aquaculture via water runoff and integrated farming.

Although no antibiotics are currently used directly for treating fish in the surveyed farms, the integration of fish farming with crop and livestock production introduces potential indirect exposure routes. Antibiotics used in livestock and horticulture can contaminate fishponds via manure fertilization, irrigation runoff, or drainage from nearby fields, thereby exerting selective pressure on aquatic microbiota. This aligns with the “One Health” perspective, emphasizing the interconnectedness of human, animal, and environmental health in driving resistance dynamics (Alhaji et al., 2024).

In contrast, resistance patterns for Cotrimoxazole, Doxycycline, Streptomycin, and Ciprofloxacin showed no statistically significant interspecies variation. These antibiotics exhibited generally low resistance percentages and clustered near the origin in the PCA biplot (Figure 3), suggesting limited contribution to the observed variance in bacterial resistance profiles. This may indicate either conserved bacterial targets or limited environmental exposure to these drugs in the study area. Ciprofloxacin, in particular, demonstrated the highest uniformity, possibly due to its mechanism of targeting essential and highly conserved bacterial enzymes such as DNA gyrase and topoisomerase IV (Rajesh et al., 2025; Vezeau and Kahn, 2025)

The PCA analysis (Figure 3) further clarified relationships among antibiotics and bacterial species. The principal component loading vectors for Amoxicillin and Erythromycin showed strong discriminatory power along the first two dimensions, effectively separating resistant species such as *E. coli*, *A. hydrophila*, and *V. harveyi* from the rest. The clustering of other species near the origin suggests shared susceptibility profiles and minimal variance in resistance to the other tested antibiotics.

These findings underscore the need for enhanced antimicrobial stewardship in aquaculture, even in contexts where antibiotics are not directly applied. Proactive monitoring of environmental exposure routes, integrated with species-specific susceptibility data, is essential for preventing the emergence and dissemination of resistance. Educational outreach to farmers about indirect antibiotic pathways and implementation of biosecurity practices can serve as effective preventative strategies.

## Conclusion

There was significant species-level variation in antibiotic resistance among fish farm bacterial isolates in Uasin Gishu, with highest resistance observed to amoxicillin and erythromycin. The results suggest indirect antibiotic exposure, likely from integrated livestock-crop systems, is driving resistance. In contrast, ciprofloxacin, doxycycline, streptomycin, and cotrimoxazole remained largely effective.

## RECOMMENDATION

To address the emerging antibiotic resistance in aquaculture environments, this study recommends enhanced farmer education on indirect exposure pathways, strict waste management practices, and responsible antibiotic use across sectors, and further research into resistance mechanisms and environmental transmission routes.

## ACKNOWLEDGEMENTS

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## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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