**Characterization of** **Antimicrobial-Resistant Non-Typhoidal Salmonella and Other Bacteria in Integrated Fish Farming Environments in Nyeri County**

**ABSTRACT**

**Aim:** This study aimed to characterize antimicrobial resistant non-typhoidal *Salmonella* and other bacteria in integrated fish farming environments in Nyeri County.

**Study Design:** Integrated fish farming systems utilizing animal manure were investigated across three sub-counties: Tetu East, Nyeri Town, and Mathira.

**Methodology:** Samples from fish, pond water, and manure were collected and processed for microbial analysis. Fish intestinal contents, water, and manure were incubated in nutrient broth and cultured on nutrient agar. Bacterial identification was done using MALDI-ToF mass spectrometry. Non-typhoidal *Salmonella* (NTS) was identified using selective culture media and confirmed through 16S rDNA typing. Antimicrobial susceptibility was tested via broth micro dilution, and Plasmid DNA of resistant isolates were screened through PCR and gel electrophoresis.

**Results:** Multiple pathogens were detected, including *Escherichia coli*, *Shigella spp.*, *Staphylococcus spp.*, *Aeromonas hydrophila*, *Serratia*. NTS was found in 42.3% of all samples. Over 88% of *Salmonella* isolates showed MIC ≤1 µg/ml for ciprofloxacin, while 75% had MIC ≥3.125 µg/ml for azithromycin. High resistance was observed for oxacillin (54% with MIC 300–6000 µg/ml) and streptomycin (50% with MIC ≥24,000 µg/ml). Resistance rates were 60.6% for ciprofloxacin, 100% for oxacillin and streptomycin, and 23.1% for azithromycin. Resistance genes detected included *parC*, *gyrA*, *msrA*, *strA*, and *strB*, though *mecA* was absent.

**Discussion and Conclusion:**The findings suggest that integrated fish farming poses a risk of transmitting antimicrobial-resistant pathogens from animal manure to aquatic environments. This not only threatens aquatic animal health but also poses a serious risk to human health through potential transmission via direct contact, environmental exposure, or consumption of contaminated fish products.

**Keywords;** Non-typhoidal *Salmonella*, Integrated fish farming, Antimicrobial Resistance

# **1.INTRODUCTION**

Fish are a vital source of essential macro and micronutrients for a healthy population. In Kenya, although the per capita consumption of fish steadily increased to 2.89 kg by year 2021, this has remained way below the FAO recommended average of 20kg year-1 person-1(Pauly & Zeller, 2017). The government has had to meet an annual deficit of 100,000 metric tons through imports from China, Korea, Japan and Uganda (Commercial, 2024). With popularization of freshwater fish farming, integrated fish culture has become common practice among small scale farmers with Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) being the most reared species (Opiyo et al., 2018).

Manure from livestock production is administered to fish ponds primarily as a source of nutrients to support the growth of photosynthetic organisms such as phytoplankton and zooplankton, which forms the base for pond’s food web (Endebu et al., 2016). Within integrated fish-livestock or fish-poultry farming systems, antimicrobials, their residues, and bacteria may enter the fish ponds through animal manure, farm water or waste animal feed, which many farmers use to supplement fish feed (Xu et al., 2020). These increase the risk of horizontal gene transfer of antimicrobial resistance (AMR) from bacteria in manure to those in aquatic ecosystem that in turn contaminate fish and subsequently humans who are the end consumers (Romero et al., 2012).

Antimicrobial resistant bacteria, including multi-drug resistant ones have been isolated in fish farms and the surrounding aquatic environments (Chuah et al., 2016) .In addition, antimicrobial residues have been found in the sediments of marine fish farms (Maki et al., 2006). Fish ponds which are rarely emptied have been found to accumulate antimicrobials and their residues (Popowska, 2021). Such a buildup could establish selective pressure that favor growth of antibiotic-resistant bacteria (Tello et al., 2012). Nevertheless, cross resistance is a common phenomenon where a bacteria resistant to one class of antibiotics often confers resistance to other antibiotics of the same group (Petersen et al., 2002). The use of antibiotics as growth promoters in animal husbandry has been linked to certain antimicrobial resistance patterns among human bacterial pathogens, suggesting that there is a possible flow of antimicrobial resistance genes between animal and human pathogens (Marshall & Levy, 2011). Potential transfer of antimicrobial resistant bacteria and AMR genes from aquaculture environments to humans may occur through direct consumption of improperly cooked fish and associated products (Cheng et al., 2021).

While non-typhoidal *Salmonella* (NTS) is known to be a major cause of diarrhea and intestinal inflammation in humans and animals (laupland, 2010), and is an important enteric pathogen rampant among children under 5 years in Sub-Saharan Africa and South Asia (Das et al., 2022), no study in Kenya has specifically investigated this in aquaculture system .The study therefore aims to isolate and characterize antimicrobial resistant non-typhoidal *Salmonella* and other bacteria in integrated fish farming systems.

# **2.0 MATERIALS AND METHODS**

# **2.1 Study design and sample collection**

 A cross-sectional study was carried out in Tetu East, Nyeri Town and Mathira sub-Counties of Nyeri County conducted in January of 2021. The sample size was calculated using Cochran formula (1963:75).

$$n=\frac{Z^{2}×P×(1-P)}{E^{2}}$$

Where n= sample size, Z= z-score, P= estimated proportion of population, E= margin of error

P=97, Z= 95%, E=5%

 n= 78

A questionnaire was administered to farmers to capture demographic data and acquire information on use of farm manure to fertilize fish ponds as well as risk factors for antimicrobial resistance. Fish samples were trapped using a fish net designed to capture mature fish only. Water samples (10ml) were collected in sterile 15ml falcon tubes while 5g of manure were collected and put in ziplock bags. The samples were aseptically packaged and transported to the laboratory in a cool box for analysis. Samples were processed within 24 hours of collection while still fresh.

# **2.2 Isolation and purification of bacteria**

Intestinal contents of the fish were extracted aseptically and incubated in nutrient broth at 37oC for 24 hours. Fresh farm manure (1g) was resuspended in phosphate buffered saline (PBS) (pH 7.2). Then, 1 ml of the manure filtrate and 1 ml of farm water were each incubated separately in 9ml of nutrient broth for 24 hours at 37oC. The overnight cultures were streaked on nutrient agar (Himedia, India) and incubated for a further 24 hours at 37oC. The isolates were sub-cultured in order to obtain single colonies for strain typing by Matrix Assisted Laser Desorption/Ionization mass spectrometry ( MALDI-TOF MS) (Shimadzu Axima Confidence, Japan).

# **2.3 Microbial strain typing by MALDI-TOF MS**

A solution of alpha-cyano-4-hydroxycinnamic acid (CHCA, matrix) at a concentration of 40 mg/ml (Sigma-Aldrich, St. Louis, USA) was prepared using LC-MS grade solvents; acetonitrile, ethanol, and water mixed in equal parts (3:3:3) with 3% trifluoroacetic acid. Briefly, using a sterile micropipette tip, 0.5 µl bacterial culture was transferred onto the target plate and each spot overlaid with 0.5 µl of 25% formic acid (SigmaAldrich St.Louis, USA). This was followed by applying 0.5µl α-CHCA matrix onto each spot and thoroughly mixing and air drying before MALDI-TOF MS analysis in Shimadzu Axima Confidence (Shimadzu, Kyoto, Japan). The characteristic MALDI-TOF mass spectra fingerprints were acquired and microbial identification was done through Spectral Archive and Microbial Identification System (SARAMIS).

# **2.4Non typhoidal *Salmonella* isolation and identification**

Isolation of non-typhoidal *Salmonella* was done using selective media. Pre-enrichment of manure, water and fish enteric samples was done in peptone water (Himedia, India) with gentle shaking for 24 hours at 37oC before enrichment in Rappaport Vassiliads broth (Himedia, India ) for a further 24 hours. Tenfold serial dilution of bacterial cultures was done followed by plating on Bismuth sulfite agar (Himedia, India) for 24 hours. Plates with characteristic round black colonies surrounded by a metallic sheen were presumed to be *Salmonella* and were considered for molecular characterization*.*

# **2.5 Molecular characterization of non-typhoidal *Salmonella***

For all the samples that turned positive for *Salmonella,* DNA was extracted using Qiagen DNA extracting kit following manufacturer’s instructions. Quality assessment of the extracted DNA was done followed by 16S rDNA gene amplification using primers specific for Non-typhoidal *Salmonella*. (F 5’-CAGCCACACTGGAACTGAGA-3’ and R 5’-GTTAGCCGGTGCTTCTTCTG-3’). The PCR reaction consisted of: 12.5µl of One Taq Quick-Load 2X Master Mix (New England Biolabs), 1µl of 10µM of each primer, 2µl of the DNA template and nuclease free water to a total volume of 25µl. The PCR conditions were as follows; Initial denaturation at 94oC for 1 min, followed by 35 cycles of denaturation at 94oC for 30sec, annealing at 55oC for 30sec, extension at 68oC for 60sec, and a final extension at 68oC for 5 min. PCR products were analyzed by 1.5% agarose gel electrophoresis against a 100bp DNA ladder (Sigma-Aldrich, USA) and visualized under a UV trans-illuminator. The expected band size was 204bp.

# **2.6 Antimicrobial susceptibility test**

All samples positive for *Salmonella* were incubated in nutrient broth for a further 24 hours at 37oC. To determine the minimum inhibitory concentration, broth micro-dilution was performed on a 96-well plate. Fifty (50µl) of the Mueller Hinton broth (MHB) was dispensed in each well of column 1-10 while Column 11 contained 100µl of the uninoculated broth (as a control) and column 12 contained 100 µl of inoculum standardized to 0.5 McFarland’s reagent. A pipette was then used to mix and transfer the different antibiotics (ciprofloxacin, azithromycin, oxacillin and streptomycin) from column 1-10, resulting in 50 µl antibiotic per well. Fifty (50) µl of the adjusted OD600 bacterial suspension was then added to the wells containing antibiotics and to the positive control well resulting in approximately 5x105CFU/ml. After incubation for 24 hours at 37oC, 20 µl of resazurin dye (0.015%) was added to all wells and the plates further incubated for 2 hours. All results were analyzed and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (2020).

#  **2.7 Molecular detection of antibiotic resistant genes**

Plasmid DNA was extracted from the resistant *Salmonella*, based on the CLSI criteria for antibiotic resistance, using plasmid DNA extraction kit (Qiagen) following the manufacturer’s instructions. Amplification was done using specific primers for detection of genes conferring resistance to oxacillin (*mecA*), azithromycin (*MsrA*), ciprofloxacin (*gyrA* and *parC*) and streptomycin (*strA* and *strB*). The primer sequences and the expected fragment size of each gene have been listed in table 1 while the cycling conditions comprised of initial denaturation temperature of 94oC for 1 minute, followed by 35 cycles of denaturation at 94oC for 30 sec, annealing at 55oC for 30 sec, extension at 68oC for 1 minute and a final extension at 68oC for 5 minutes. PCR products were analyzed by 1.5% agarose gel electrophoresis against a 100bp DNA ladder (Sigma-Aldrich, USA) and visualized under a UV trans-illuminator.

**Table 1:- Primer sequences targeting various AMR genes in plasmid DNA**

|  |  |  |
| --- | --- | --- |
| **Target gene** | **Primer sequences** | **Expected fragment size (bp)** |
| *MecA* | F 5’- TCCAGATTACAACTTCACCAGG-3’R 5’- CCACTTCATATCTTGTAACG-3’ | 162 |
| *MsrA* | F 5’-TCGCTATGGGCTGTTTCTG-3’R 5’- AACTGATAACGGCAGGATCG-3’ | 180 |
| *gyrA* *parC*  | F 5’- TACCGTCATAGTTATCCACGA-3’R 5’- GTACTTTACGCCATGAACGT-3’F 5’- CTATGCGATGTCAGAGAGCTGG-3’R 5’-TAACAGCAGCTCGGCGTATT-3’ | 313267 |
| *strA* *strB* | F 5’- CCAATCGCAGATAGAAGGC-3’ F 5’-CTTGGTGATAACGGCAATC-3’F 5’- GGATCGTAGAACATATTGGC-3’R 5’- ATCGTCAAGGGATTGAAACC-3’ | 540499 |

# **2.8 Data analysis**

Data analysis was done using Statistical Package for Social Scientists(SPSS) (version 25.0). Descriptive statistics was used to summarize demographics of fish farming. Qualitative and quantitative data values were expressed as frequencies along with percentages.

# **3.RESULTS**

# **3.1 Demographics of Fish farming**

A total of 78 samples comprising of fish samples, manure and water were collected from 25 farms. The demographic data indicated that most farmers (32%) used reserved rain water in the fish ponds, 28% used water from local community-based water projects, 16% alternately used both rain and river water,8% used water directly from the river, while another 8% used water from the county water project. Four percent (4%) used dam water while another (4%) used underground water (Figure 1).

**Figure. 1** Source of water for fish ponds.

 Majority (56%) of the farmers used commercial feeds. Only 32% of them used a mixture of commercial feed and vegetables while 8% used self-formulations comprising mainly of bran, sunflower and dairy meal. Four percent (4%) used food leftovers (Table 2).

**Table 2:-Types of Fish Feed Used by Farmers**

|  |  |  |
| --- | --- | --- |
| **Type of Feed Used** | **Number of Farmers** | **Percentage (%)** |
| Commercial feeds | 14 | 56% |
| Commercial feeds + vegetables | 8 | 32% |
| Self-formulated feed (bran, sunflower, dairy meal) | 2 | 8% |
| Food leftovers | 1 | 4% |

The study revealed 48% of the farmers applied raw manure directly into fish ponds. Of these, 71% applied only once at inception of fish rearing while 14% applied biannually. Only 7% applied annually. Fifty percent (50%) of farmers used manure from cattle while 36% used manure from poultry. Sixty-eight percent (68%) of farmers used raw (unprocessed) manure while 32% used processed manure. A few farmers (14%) used urea to fertilize their ponds (Figure 2).

**Figure 2**. Manure use among fish farmers

Interestingly, majority of farmers (52%) in this study had basic understanding of antimicrobial resistance with only 12% of them having used antibiotics in their animal husbandry or poultry rearing (Table 3).

 **Table 3:-Farmer Knowledge and Antibiotic Usage**

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Number of Farmers (n=25)** | **Percentage (%)** |
| Farmers with basic understanding of AMR | 13 | 52% |
| Farmers without understanding of AMR | 12 | 48% |
| Farmers who have used antibiotics | 3 | 12% |
| Farmers with no antibiotic use reported | 22 | 88% |

# **3.2 Microbial strain typing by MALDI TOF MS**

Among the identified bacteria, *Shigella sp*. was the most prevalent at 19.3% with *Escherichia coli* and *Enterobacter sp.* having incidences of 17.0% each. *Stapylococcus sp*. and *Serratia marcescens* had prevalence of 9.1% each. On the other hand, the prevalence of *Klebsiella pneumonia*e was recorded at 4.5% while *Aeromonas hydrophilla* had a prevalence of 3.4%. Other bacteria species identified from the samples had prevalence of <3% each. These included; *Citrobacter freundii, Rahnella aqualitis*, *Finegoldia magna*, *Arcanobacter bernadiae, Microsporum gypseum, Rhodococcus intermedius, Actinobacillus seminis*, *Neisseria animaloris, Providencia rettgeri, Lactobacillus rhamnosus, Norcadia brasiliensis, Norcadia brasiliensis, Clostridium difficile* and *Proteus mirabilis.*

# **3.3 Prevalence of Non- typhoidal *Salmonella***

Following bacterial culture on Bismuth Sulfite Agar, black colonies with metallic sheen were presumed to be *Salmonella.* A total of 42.3% (n=78) *Salmonella* isolates were recovered from fish, manure and farm water samples collected. The prevalence of Non-tyhoidal *Salmonella* in the fish samples was 34.6% while water and manure had prevalence of 5.1% and 2.6% respectively (table 4).

**Table 4:- Prevalence of Non-typhoidal *Salmonella* in fish, farm water and manure.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Types of Samples** | **No. of Samples** | **Positive for *Salmonella*** | **Prevalence (%)** |
| Fish | 58 | 27 | 34.6 |
| Farm water | 13 | 4 | 5.1 |
| Manure | 7 | 2 | 2.6 |
| **Total samples** | 78 | 33 | 42.3 |

# **3.4 Molecular characterization of non-typhoidal *Salmonella***

Genomic DNA was extracted from samples that turned positive for *Salmonella* on Bismuth sulfite agar. Non-typhoidal *Salmonella* specific primers targeting 16S rDNA genes were used for identification and the expected product of 204bp was obtained on the agarose gel (Figure 3).



**Figure 3.** Gel image of PCR products (204bp) of Non-typhoidal *Salmonella* 16S rDNA ran on 1.5% agarose gel at 50 volts for 45 minutes. M\_100bp DNA ladder(ThermoFisher), F\_ Fish sample**,** M\_ manure, W\_ water

# **3.5 Determination of minimum inhibitory concentration**

Minimum Inhibitory Concentration (MIC) of ciprofloxacin was carried out at a concentration gradient of 0.125 to 32µg/ml. This concentration is within range determined by similar studies. Majority of the isolates had MIC ≤1µg/ml, with 17 isolates (45.9%) having MIC of 1µg/ml. Two isolates (5.4%) were found to have an MIC of 0.125µg/ml, while 4 of the isolates (10.8%) had an inhibitory concentration of 0.25µg/ml of ciprofloxacin per ml. The concentration of 0.5µg/ml was recorded in 7 isolates (18.9%) and the remaining 4 samples (10.8%) had an MIC of 2µg/ml. No inhibition was observed at the highest concentration 32µg/ml of ciprofloxacin examined. For azithromycin, a concentration range between 0.1 to 50µg/ml was used. From the results, majority of the isolates had an MIC of 3.125µg/ml representing 43.2% of the total isolates with 8 isolates (21.6%) having an MIC of 1.5625µg/ml while the other 8 isolates exhibiting an inhibitory concentration of 6.25µg/ml. The MICs of oxacillin was the highest among the four drugs under study, with a concentration range of 48.125 to 24,000µg/ml. The highest MIC was 12000µg/ml with majority of the isolates (51.4%) having MIC between 3000 and 6000µg/ml. Three isolates (8.1%) showed the lowest MIC at 192.5µg/ml. For streptomycin, majority of the isolates had an MIC of ≥ 24,000µg/ml. Two isolates had an MIC of 1500 µg/ml while only 1 isolate was found to have an MIC of 12,000 µg/ml (Table 5 )

**Table 5:-MIC Distribution of Antibiotics Against Bacterial Isolates**

|  |  |  |  |
| --- | --- | --- | --- |
| Antibiotic | MIC (µg/ml) | No. of Isolates | Percentage (%) |
| Ciprofloxacin | 0.125 | 2 | 5.4 |
| Ciprofloxacin | 0.25 | 4 | 10.8 |
| Ciprofloxacin | 0.5 | 7 | 18.9 |
| Ciprofloxacin | 1 | 17 | 45.9 |
| Ciprofloxacin | 2 | 4 | 10.8 |
| Ciprofloxacin | ≥32 (No inhibition) | 3 | 8.1 |
| Azithromycin | 1.5625 | 8 | 21.6 |
| Azithromycin | 3.125 | 16 | 43.2 |
| Azithromycin | 6.25 | 8 | 21.6 |
| Azithromycin | Others | 5 | 13.6 |
| Oxacillin | 192.5 | 3 | 8.1 |
| Oxacillin | 3000–6000 | 19 | 51.4 |
| Oxacillin | 12000 | 1 | 2.7 |
| Oxacillin | Others (48.125–24000) | 14 | 37.8 |
| Streptomycin | 1500 | 2 | 5.4 |
| Streptomycin | 12000 | 1 | 2.7 |
| Streptomycin | ≥24000 | 33 | 89.2 |

# **3.6 Molecular detection of antibiotic resistant genes**

Resistance markers for three out of the four antibiotics tested were detected in the isolates comprising of azithromycin (*msrA*), ciprofloxacin (*parC* and *gyrA*) and streptomycin (*strA* and *strB*) (Figure 4). Oxacillin resistant gene (*mecA*) was not detected in any of the isolates.



499bp

540bp

**Figure. 4**. Gel image of multiplex PCR of *strA* (540bp), *strB* (499bp), *parC* (267bp), *msrA* (180bp), *gyrA* (313bp), *mecA* (162bp) genes ran on 1.5% agarose gel at 100 volts for 30min. M\_100 bp DNA ladder (ThermoFisher), W\_ water, M12\_ Manure, F\_ Fish NC\_ Negative control

# **4. DISCUSSION AND CONCLUSION**

# **4.1 Discussion**

The findings of this study indicated that majority of the ponds were indeed contaminated with potential fish pathogens and non-fish pathogens with more than 98% of the microorganisms isolated comprising of bacteria with less than 2% being fungi. Similar findings were reported by Samuel et al., (2019). However, there was variation in the prevalence of *Shigella sp*. (19.3%) and *Enterobacter sp* (8.3%.). A similar study conducted in Tanzania reported no significance difference in prevalence of bacteria isolated from fish with *E.coli* being the most predominant at 39%, followed by *Salmonella sp*.(16%), *Staphylococcus sp*.(8%), *Citrobacter* (4%), *Shigella sp*.(3%) and *Pseudomonas sp*.(1%) (Marijani, 2022). In another study in India, 163 gram-negative bacteria were isolated from aquatic environments with multiple antibiotic resistance of above 50% (Mohanta & Goel, 2014).

Presence of *E.coli* in the pond environment was an indicator that water quality in the fish ponds was unsuitable for fish rearing. *Salmonella enterica* is a zoonotic foodborne pathogen of medical significance that is easily transmitted along the food chain, as identified by the Centres for Disease Control and Prevention (CDC) (Safety & Practices, 2021). In Kenya, Salmonellosis is the second to pneumococcal disease as leading cause of mortality in children under five years (Muthumbi et al., 2015). Several outbreaks of sea food related salmonella have been documented elsewhere (Kumar et al., 2015). As *Salmonella* is naturally not a fish pathogen, its presence in aquatic systems is most likely due to introduction of fecal microbes into culture ponds. In a study previously conducted in Kenya, Kimera et al ( 2020 ) showed a significant prevalence of *Salmonella enterica* in pigs, chicken, eggs and cows of which 40% of the isolates were resistant to one or more antibiotics. In this study, the prevalence of *Salmonella enterica* was 42.3%. Of the individual sample categories, 34.6% of fish samples, 5.1% of pond water and 2.6% of manure had *Salmonella*. This study is in agreement with study conducted by (Hamdi et al., 2007) which indicated a prevalence of 48% of *Salmonella* in aquatic fish in Bouira, Algeria. On the contrary, a study conducted by the University of Benin, Nigeria (Beshiru et al., 2019) reported a prevalence of 14.6% in fish samples collected from aquatic fish farms. However, this study could not point out a direct relationship between the use of manure and *Salmonella* contamination of fish ponds, possibly due to the small number of manure samples collected from farmers. Based on demographic data, only 7/25 farmers (28%) had used manure in the recent past, of which only 2.6% of the samples were positive for *Salmonella*. Most farmers who used manure applied once at inception of fish rearing some 1-3 years before this study was carried out. This disparity in prevalence could be due to differences in hygienic practices by farmers, particularly in the preparation of feed formula. Our study identified excessive algal bloom in some ponds due to high nutrition from food and feed left overs released into ponds which could potentially promote proliferation of bacteria, including antimicrobial resistant bacteria.

Using the CLSI 2012 revised breakpoints for MIC, 60.6% (20/33) were resistant to ciprofloxacin. However, 14/33 (42.4%) of the isolates showed intermediate resistance. These results denote an increase in resistance from previous studies. Since 1994 there has been increase in the occurrence of resistance to ciprofloxacin of *Salmonella enterica* with a propensity to cause gastrointestinal infections in humans (Thre et al., 1999). A study in Ghana of *Salmonella* prevalence in humans showed a lower resistance (25.0%) to ciprofloxacin (Andoh et al., 2017). In a more recent study in Taiwan, a lower resistance of non typhoidal *Salmonella* to ciprofloxacin was also recorded (Chang et al., 2020). A significantly higher resistance rate of 39% was recorded in a study by Lin et al., (2015) to characterize fluoroquinolones resistant *Salmonella* strains isolated from food samples. In this study, the MIC for ciprofloxacin was established to be ≤ 1µg/ml .In Nepal, MIC range of 0.5 to 1 µg/ml was observed for ciprofloxacin (Khanal et al., 2017).On the contrary, other studies have reported higher MIC (>8 µg/ml) for ciprofloxacin (Vidovic et al., 2019). Some of the isolates (48.5%) were 100% susceptible to azithromycin. Contrary to our study, a resistance rate of 25% to azithromycin has been recorded in other studies (Lin et al., 2015). Our results are consistent with the study by Gunell et al.,(2010) which reported azithromycin MIC of 4 to 8 µg/ml. A similar study conducted in Nepal (Khanal et al., 2017) reported MIC range of 0.125 to 2.0 µg/ml.

At the same time, this study revealed 100% resistance to oxacillin. Even though majority of the isolates did not show distinct MIC for Streptomycin, some isolates had MIC of between 125µg/ml and 800 µg/ml in agreement with the results reported by (Edrington et al., 2002). This study established that 100% of the isolates had resistance to streptomycin. This differed from a study in Ethiopia to determine the resistance to the drug in animals and humans and reported a resistance rate of 47% (Id et al., 2020). More than 60% of the isolates showed resistance to more than one antibiotic.

Five out of the 6 targeted antibiotic resistance genes were detected in the isolates. There were plasmid associated genes, an indication that resistance could be plasmid-mediated. However, no *mecA* gene was detected in any of the isolates despite a high MIC for oxacillin being recorded. This could be attributed to the fact *mecA* is mainly associated with resistance against oxacillin in *Staphylococcus sp* or indicates a different mechanism of resistance by the target bacteria.

# **4.2 Conclusion**

The findings of this study provide compelling evidence that integrated fish farming systems in Nyeri County serve as critical hotspots for the development and propagation of antimicrobial-resistant (AMR) bacteria. The isolation of resistant strains, most notably Non-typhoidal Salmonella, from fish, pond water, and manure samples demonstrates the extent to which aquaculture environments are becoming reservoirs of resistance. This does not only threaten aquatic animal health but also poses a serious risk to human health through potential transmission via direct contact, environmental exposure, or consumption of contaminated fish products. A key contributor to this growing problem is the widespread, and often unregulated, use of antibiotics in fish farming frequently driven by limited access to veterinary guidance and a lack of awareness among farmers about the consequences of misuse. Furthermore, inadequate waste management practices and the integration of livestock manure into fish ponds exacerbate the persistence and dissemination of resistant bacteria within these ecosystems. These insights underscore the urgent need for a multi-sectoral response rooted in the One Health approach, which recognizes the interconnectedness of human, animal, and environmental health. Strengthening antibiotic stewardship, enhancing farmer education, and enforcing stricter regulations on antibiotic usage are crucial interventions. Additionally, there is a need to invest in routine AMR surveillance within aquaculture systems, promote alternative disease control strategies such as probiotics and vaccination, and encourage the adoption of sustainable farming practices.

# **DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

The authors declare that NO generative AI technologies such as Large Language Models and text-to-image generators have been employed during writing and editing this manuscript.

#  **ETHICAL APPROVAL**

Ethical approval to conduct the research was sought out from the Kenyatta National Hospital/University of Nairobi Ethics Review Committee (P228/04/2021) and National Commission for Science, Technology and Innovation (NACOSTI/P/21/13314).

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