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Antibiotic Resistant Pathogenic Bacteria Isolated from Aquaculture Systems in Bungoma County, Kenya

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Abstract Aquaculture production in Kenya has been growing exponentially as a Government initiative to meet population nutritional requirements and food security. Unfortunately factors exist such as fish infection and disease that work against the health and survival of fish in aquaculture. This study focused on identifying bacterial pathogens present in aquaculture systems in Bungoma County and determined how the pathogens respond to commonly used antimicrobial agents. During the study, *Vibrio vulnificus*, *Vibrio parahaemolyticus*, *Aeromonas hydrophila* and *Pseudomonas aeruginosa* were recovered from farmed Nile tilapia while *Aeromonas hydrophila* and *Streptococcus iniae* were isolated from fish source pond water and fish feeds respectively. Among the bacterial isolates from Nile tilapia, *Vibrio vulnificus* and *Aeromonas hydrophila* were resistant to ampicillin while *Vibrio parahaemolyticus* and *Pseudomonas aeruginosa* were resistant to cefuroxime and ampicillin. *Aeromonas hydrophila* recovered from pond water were found to be resistant to both ampicillin and cefuroxime whereas, *Streptococcus iniae* isolated from fish feeds were observed to be resistant to ceftazidime, cefepime and nalidixic acid, which is a warning that unless we find alternative antimicrobial agents the aquaculture industry is likely to collapse. When the bacterial isolates were subjected to PCR, all five bacterial pathogens isolated from fish, pond water and fish feeds were found to contain bla_{TEM} gene amplified at 424bp.

Keywords: *antibacterial agents, fish diseases, vibrio, aeromonas, pseudomonas, and streptococcus, molecular analysis and* bla_{TEM} *gene*

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1. Introduction

When farmed fish get infected with bacterial pathogens, they are often treated with a variety of drugs available on the market. But these drugs may not terminate the disease and/or are excreted from fish, and end up interacting with soils, enter the food chain by plant uptake, leach into groundwater and find their way into surface water through runoff and drain flows [1,2,3].

The drugs available on the market include antimicrobials (against bacteria and fungi), antiparasitics, anaesthetics, and anticoccidials, besides vaccines. The antimicrobials used to treat bacterial infections are further grouped as; penicillins (penicillins, amoxillin, ampicillin and ampicillin-subacttum), β-lactams (cefepime, cefuroxime), tetracyclines (doxycycline, tetracycline and oxytetracycline), cephalosporins (cephalexin, cefotaxime and ceftazidime) and carbapenems (imipenem). Others are quinolones and fluoroquinones (ciprofloxacin, nalidixic acid, ofloxacin and levofloxacin), aminoglycosides (amikacin, gentamicin and kanamycin), phenicols (chloramphenicols), macrolides (erythromycin, clarithromycin and azithromycin) as well as folote pathway inhibitors (trimethoprim-sulfamethoxazole) [4,5]. The antimicrobials treatment of infected fish is principally based on a minimum inhibitory concentration (MIC). The MIC is the lowest concentration of a drug which prevents visible growth of bacterium after overnight incubation [6]. The MIC for each drug is further classified as S, I, R; where R=resistant, I=intermediate and S=susceptible based on established interpretation guidelines [7,8]. Again, each category (S, I & R) has an MIC break-point. The break-point is a cutoff for each interpretation category established and the break-points are specific to specific bacteria and drug [7].

Globally, there is limited data on antimicrobial resistance of bacteria present in aquaculture systems [9,10]. Yet, it is documented that indiscriminate use of antimicrobials in aquaculture may lead to emergence of resistant strains of bacteria infecting farmed fish [12,13]. This is because some of these antimicrobials especially flouroquinoles are known to persist in the environment after long term use [14]. Therefore, use of antimicrobials

in the treatment of infected farmed fish should be monitored frequently in order to evaluate the emergence and spread of pathogenic bacterial resistance. *Vibrio vulnificus* isolated from oysters of Louisiana Gulf in USA were found susceptible to ciprofloxacin, gentamicin, cefotaxime and ceftazidime as well as ampicillin [15] while *Vibrio vulnificus* isolates from German coastal waters were found susceptible to nalidixic acid, ampicillin, cefotaxime and Ceftazidime while resistant to amikacin and gentamicin [16]. On the other hand, *Vibrio parahaemolyticus* isolated from Korean seafood were observed to be resistant to cefotaxime and ceftazidime [17] whereas, *Vibrio parahaemolyticus* resistant to ampicillin, amikacin, cefotaxime and ceftazidime were recorded for isolates recovered from shellfish in Selangor, Malaysia [18].

Aeromonas hydrophila isolated from fish and crabs in Western Australia were susceptible to amikacin, cefepime, ciprofloxacin, gentamicin, ceftazidime and nalidixic acid [19] while, *Aeromonas hydrophila* isolated from sea cucumber, bivalves and sea sediments in Melaka in Malaysia were resistant to ampicillin and nalidixic acid [20]. Further, *Aeromonas hydrophila* isolated from wastewater in Eastern Cape Province, South Africa were resistant to ampicillin but susceptible to gentamicin, cefotaxime, ciprofloxacin and nalidixic acid [21]. Locally, the bacterium has been recovered from River Njoro in Nakuru County (wild aquatic environment) and found to be resistant to ampicillin, gentamicin and ceftazoxime but sensitive to ciprofloxacin, nalidixic acid ceftazidime and cefotaxime [22]. The *Pseudomonas aeruginosa* isolated from Armenian fish farms was found to be resistant to ampicillin [12]. On the other hand, *Streptococcus iniae* sampled from fish farms in Jeju Island, Korea were recorded as being susceptible to cefotaxime [23].

Bacteria are known to trigger mechanisms to resist drugs either biochemically or genetically. Biochemically, bacteria resist antibiotics by inactivating drugs with enzymes; reducing drug access to sites of action through cell wall thickening; altering the drugs target so that antibacterials no longer binds to it; bypassing drug's metabolism of working; and developing tolerance. On the other hand, genetic mechanism involves acquiring and expressing antibiotic resistance genes [24,25,26]. Antibiotic resistance pattern among the bacterial strains may be varied depending on the place of origin of the strains [27]. Some of the known antibiotic resistance genes are *aadA*, *strA* and *strB* which confer resistance to aminoglycosides; *sul1*, *sul2* and *sul3* which confer resistance to sulphonamides; *floR* and *cmlA* genes conferring resistance to phenicols. Other genes are AmpC-types (*CMY*, *FOX*, *MOX* and *LAT*), oprL (*gyrA* and *gyrB*), *ermB* and β-lactams (*TEM*, *SHS* and *CTX-M*) that are known to confer resistance to lactams [28,29].

The β-lactam genes encode enzymes β-lactamases which catalyse the hydrolysis of amide bond of β-lactam ring present in the β-lactam antibiotics, helping the spread of β-lactam resistant bacterial strains [28]. The bla_{TEM} gene encoding antibacterial resistance may be placed on transferable elements such as plasmids, integrons and transposons [30]. However, the β-lactam genes were originally located on the bacterial chromosome [31,32]. Multiple antibiotic resistances is associated with plasmids in many species of bacteria [33]. The bla_{TEM} genes are prevalently plasmid-mediated in Gram negative bacteria and the occurrence could be as high as 61% [34, 35].

2. Objective of the Study

The objectives of the study were to; (i) determine sensitivity of pathogenic bacteria isolated from pond water, fish feeds and Nile tilapia in Bungoma County against selected antibacterial agents, and (ii) determine the relationship between the antibacterial resistance phenotypes and the genomic antibacterial resistance genes present in the pathogenic bacteria isolated from pond water, fish feeds and Nile tilapia grown in aquaculture systems in Bungoma County.

3. Materials and Methods

3.1. Sensitivity Tests

The antimicrobials such as amikacin (AK), ampicillin (AX), cefepime (CPM), cefotaxime (CTX), cefuroxime (CXM), ceftazidime (CAZ), ciprofloxacin (CIP), gentamicin (GEN), and nalidixic acid (NA) (Himedia) which were tested are commonly used antibiotics. The isolated and identified pathogenic bacteria [36] that had been stored in double strength nutrient broth (added glycerol) were subcultured on nutrient agar and incubated for 24h at 30^0C. Thereafter, using a sterile wireloop, each bacterial strain was spread on Mueller-Hinton agar (Himedia) plate. Impregnated antimicrobials on a disc at a concentration of 30 µg for each disc were placed on each prepared Mueller-Hinton agar (Himedia) plate containing bacterial strains and incubated for 24h at 30 ℃. After 24h, the diameter of developed clear zones of each disc were measured in millimetres (mm) and recorded. The diameter of clear zones of each disc was classified for each drug as resistant, intermediate or susceptible depending on the break-points.

3.2. Molecular Analysis

The isolated pathogenic bacteria (*Aeromonas hydrophila*, *Vibrio vulnificus*, *Vibrio parahaemolyticus*, *Pseudomonas aeruginosa* and *Streptococcus iniae*) that had been refrigerated were taken to the National Museums of Kenya Genetic Laboratory. The isolates were subjected to genotypic analysis using self-designed oligonucleotide primer bla_{TEM} gene based on nucleotide sequence of the bla_{TEM} gene listed in National Centre for Biotechnology Information (NCBI) GenBank Database. The ampicillin resistant *Escherichia coli* sourced from Kenyatta University Microbiology Laboratory was used as a control. The *Escherichia coli* is known to resist ampicillin 100% due to presence of bla_{TEM} gene that is amplified at 424bp [37].

4. Results

The pathogenic bacteria recovered during this study when subjected to selected antibacterial agents, had varying diameter value ranges. The values are

classified as S, I, R; where R=resistant, I=intermediate or S=susceptible based on CLSI interpretation guidelines.

4.1. Sensitivity of Pathogenic Bacteria Present in Nile Tilapia

The four pathogenic bacteria recovered from Nile tilapia were susceptible to tested antibacterial agents with different diameter value ranges (Figure 1). *Vibrio vulnificus* which was isolated from fish scales (Plate 1) sampled from Bungoma South Sub County had diameter ranges of 22mm to 5mm. *Vibrio vulnificus* recovered from skin samples from Bungoma West (Plate 2) had diameter ranges of 19mm to 30mm. The bacterium was found to be susceptible to amikacin, ceftazidime, ciprofloxacin, cefuroxime, cefotaxime, cefepime, gentamicin and nalidixic acid. *Vibrio parahaemolyticus* isolated from gills from Bungoma North samples (Plate 3) was found susceptible to amikacin, ceftazidime, ciprofloxacin, cefotaxime, cefepime, gentamicin and nalidixic acid with diameter ranges between 9mm and 29mm.

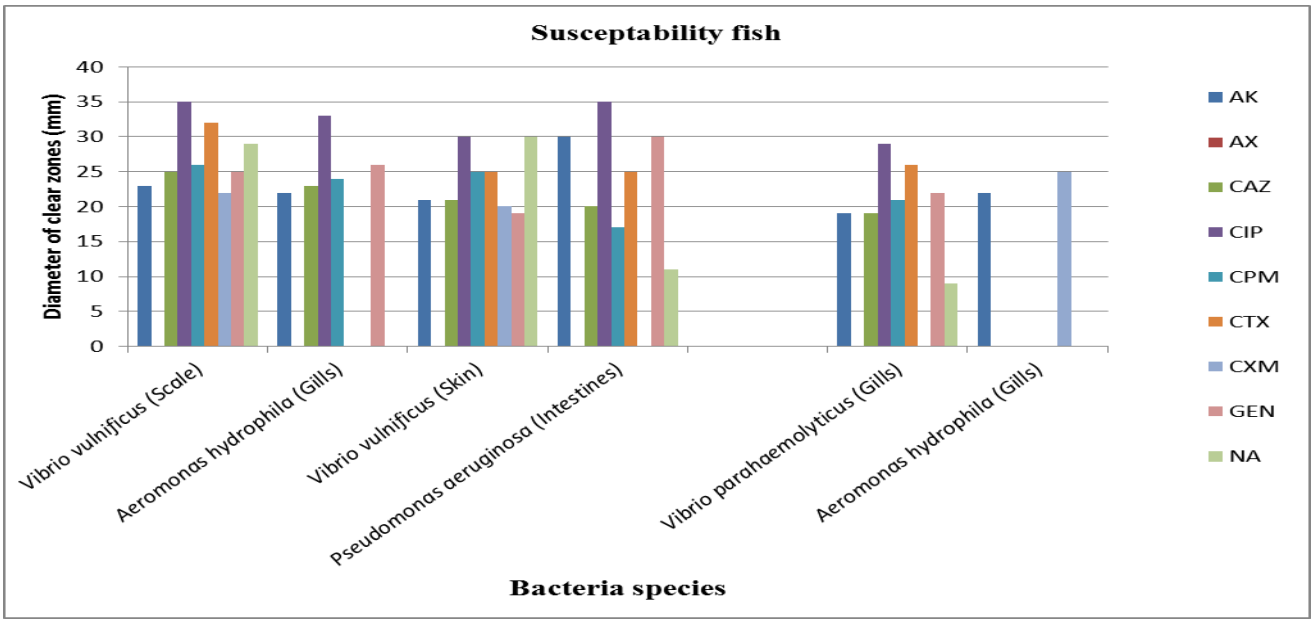

Figure 1. Antimicrobial susceptibility responses for bacterial isolates from Nile tilapia (AK-Amikacin, AX-Ampicillin, CAZ-Ceftazidime, CIP-Ciprofloxacin, CXM-Cefuroxime, CTX- Cefotaxime, CPM-Cefepime, GEN-Gentamicin, and NA-Nalidixic acid)

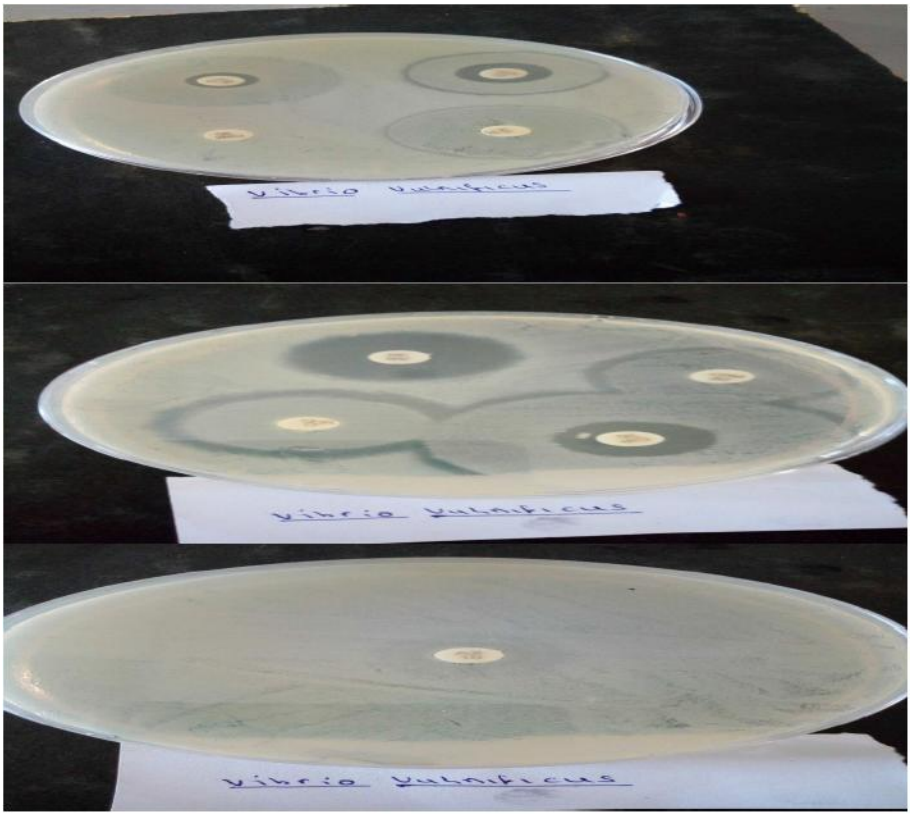

Plate 1. Impregnated sensitivity test for *Vibrio vulnificus* recovered from scales in fish from Bungoma South Sub County

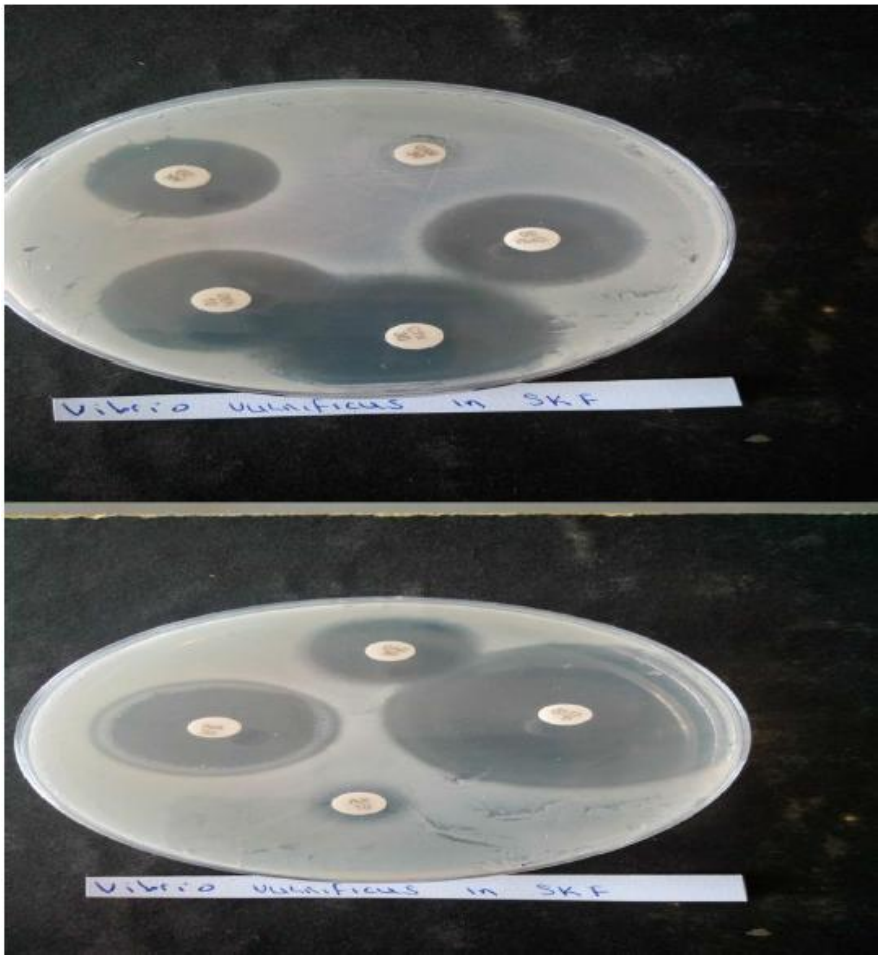

Plate 2. Impregnated sensitivity test for V. vulnificus recovered from skin from fish in Bungoma West Sub County

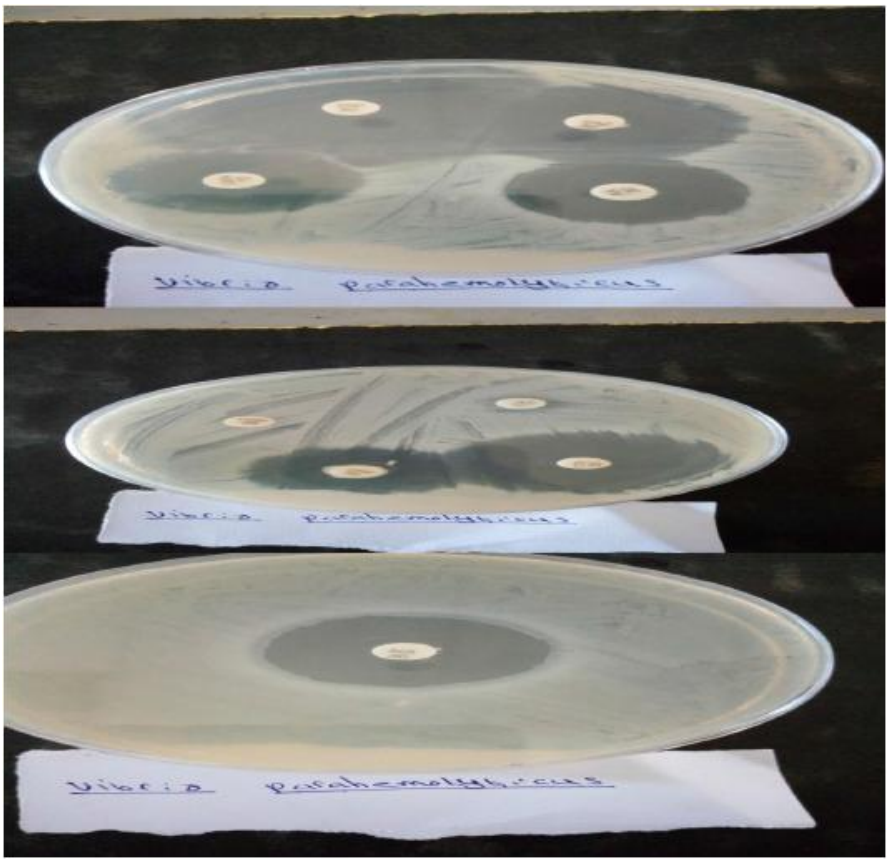

Plate 3. Impregnated sensitivity tests for *Vibrio parahaemolyticus* recovered from gills in Bungoma North Sub County

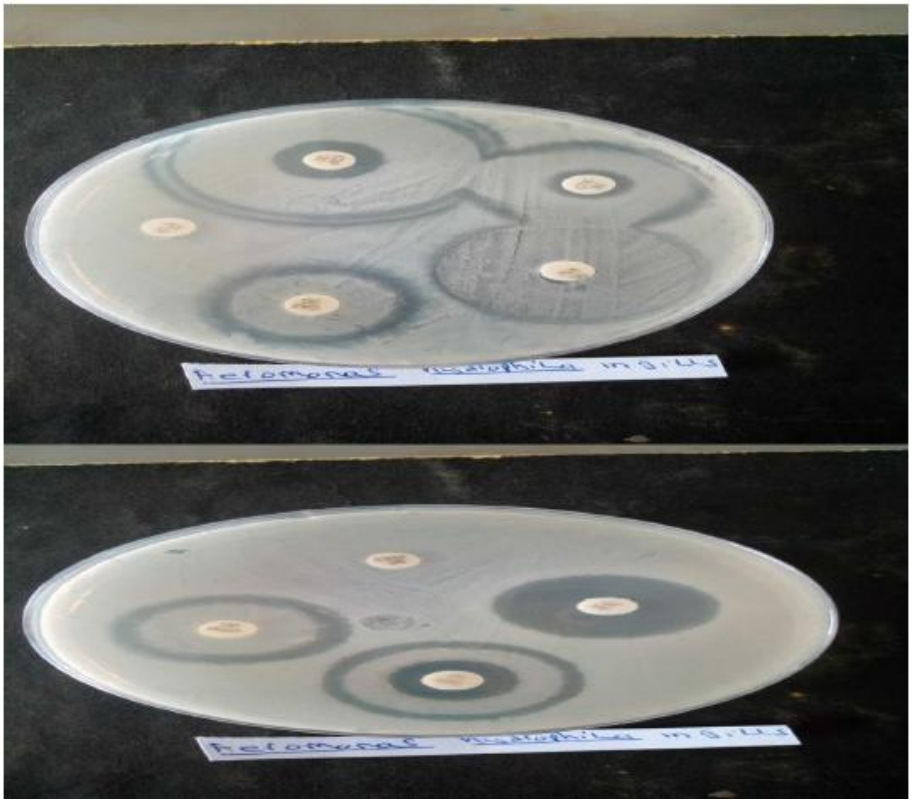

Plate 4. Impregnated sensitivity test for *Aeromonas hydrophila* recovered from gills in fish from Bungoma South Sub County

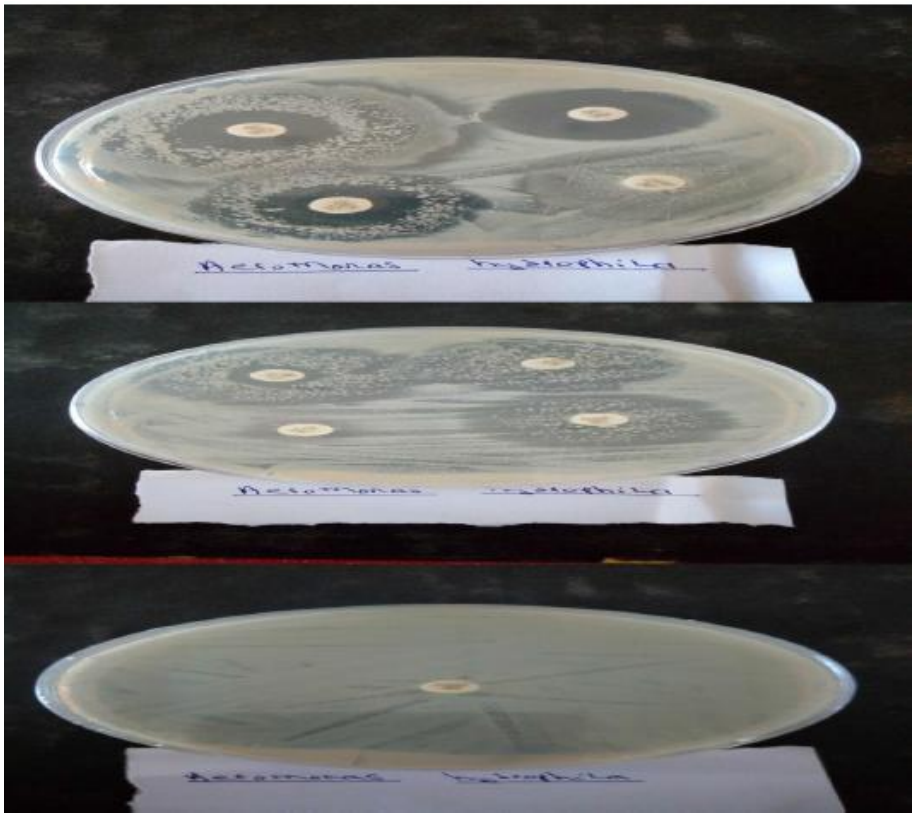

Plate 5. Impregnated sensitivity test for *Aeromonas hydrophila* recovered from gills in fish from Bungoma South Sub County

Aeromonas hydrophila isolated from gills (Plate 4) for samples from Bungoma East Sub County was found to be susceptible to amikacin, ceftazidime, cefepime, cefuroxime and nalidixic acid with diameter range from 22mm to 26mm. Further, *Aeromonas hydrophila* also isolated from gills (Plate 5) but from Bungoma South Sub County samples was susceptible to amikacin, ceftazidime, ciprofloxacin, cefepime and gentamicin with diameter ranges of 22mm to 33mm. *Pseudomonas aeruginosa* isolated from fish intestines (Plate 6) samples from Bungoma West Sub County was susceptible to amikacin, ceftazidime, ciprofloxacin, cefepime, cefotaxime,

gentamicin and nalidixic acid with diameter ranges of 11mm to 35mm. *Aeromonas hydrophila* isolated from gill sample from Bungoma East Sub County was observed to have intermediate sensitivity (moderately susceptible) towards ciprofloxacin, cefotaxime and gentamicin with ranges from 27mm to 40mm (Figure 2). However, *A. hydrophila* recovered in gills from Bungoma South Sub County samples was found to be moderately susceptible (intermediate) to cefotaxime, cefuroxime and nalidixic acid with ranges of 24mm to 32mm.

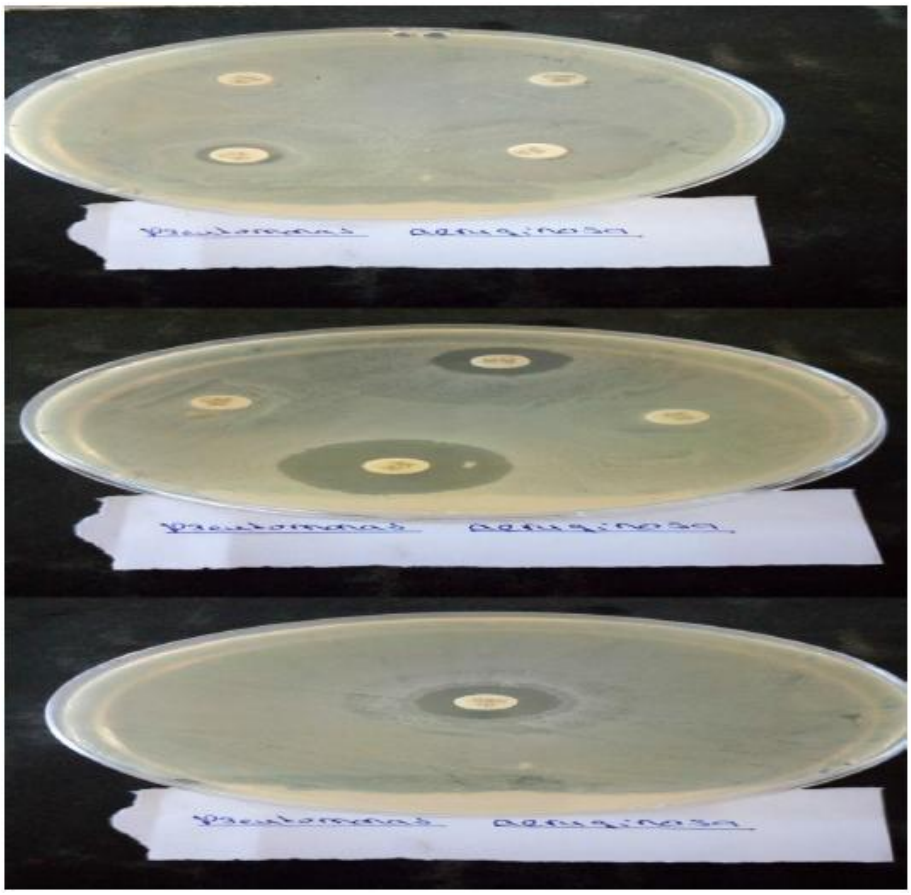

Plate 6. Impregnated sensitivity test for *Pseudomonas aeruginosa* recovered from intestines in fish from Bungoma West Sub County

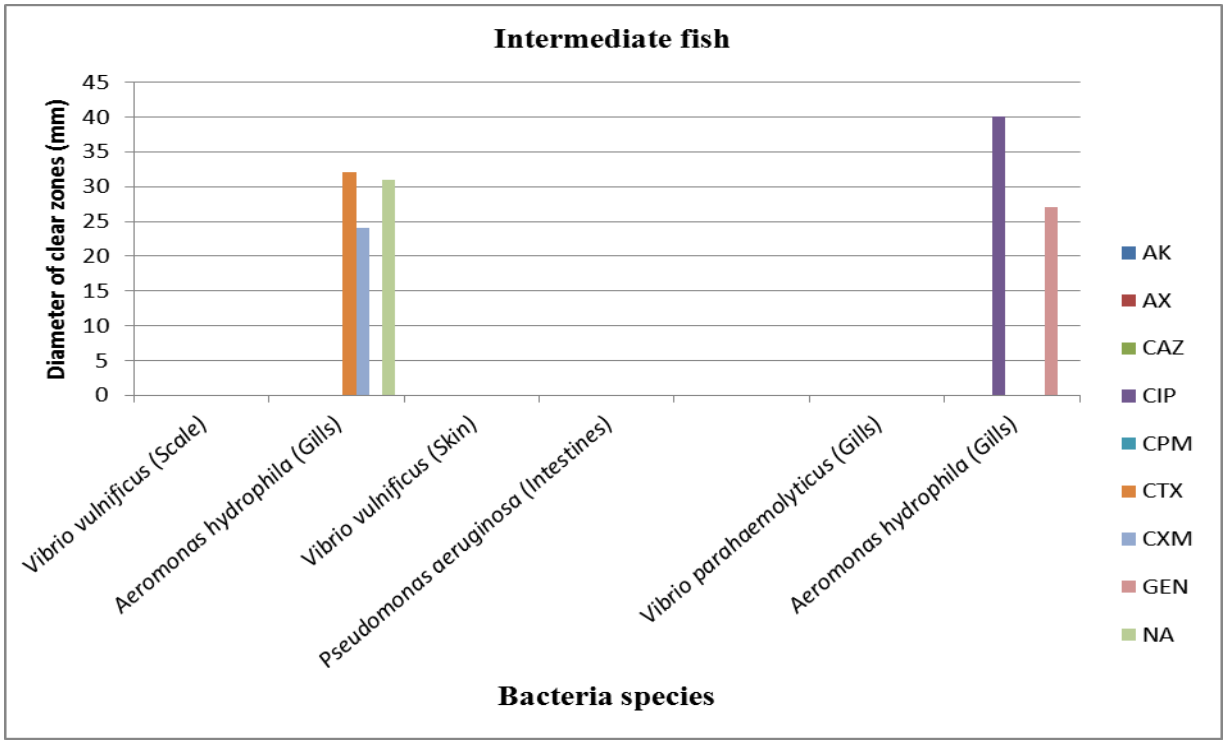

Figure 2. Antimicrobial intermediate sensitivity responses for bacterial isolates from Nile tilapia (AK-Amikacin, AX-Ampicillin, CAZ-Ceftazidime, CIP-Ciprofloxacin, CXM-Cefuroxime, CTX-Cefotaxime, CPM-Cefepime, GEN-Gentamicin, and NA-Nalidixic acid)

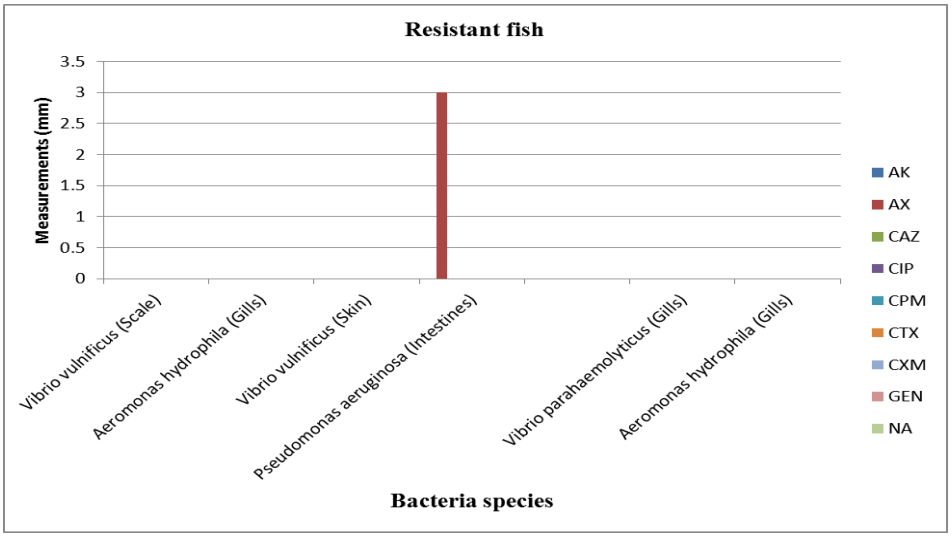

Figure 3. Antimicrobial resistant sensitivity for bacterial isolates from Nile tilapia (AK-Amikacin, AX-Ampicillin, CAZ-Ceftazidime, CIP-Ciprofloxacin, CXM-Cefuroxime, CTX- Cefotaxime, CPM-Cefepime, GEN-Gentamicin, and NA-Nalidixic acid)

The four bacterial pathogens; *Vibrio vulnificus*, *Vibrio parahaemolyticus, Aeromonas hydrophila*, and *Pseudomonas aeruginosa* isolated from Nile tilapia were found resistant to ampicillin and cefuroxime (Figure 3). *Vibrio vulnificus* that was isolated from scales of fish samples from Bungoma South and the skin for samples from Bungoma West was found to resist ampicillin with a diameter range of 0mm. *Vibrio parahaemolyticus* isolated from fish gill samples from Bungoma North Sub County was resistant to ampicillin and cefuroxime with a diameter range of 0mm for both agents. Further, *Aeromonas hydrophila* isolated from gills of fish sampled from Bungoma East Sub County and Bungoma South Sub County were resistant to ampicillin with a diameter range of 0mm. Besides, *Pseudomonas aeruginosa* that was isolated from intestines in fish sampled from Bungoma West sub County was resistant to ampicillin with a diameter range of 3mm and cefuroxime with a diameter range of 0mm. Overall, it was found that there was no significant difference in resistance of different pathogenic bacteria recovered from Nile tilapia (P=0.087). There was no significant difference among the tested agents across the sub counties (P=0.189).

4.2. Responses of Pathogenic Bacteria Present in Fish Ponds

Aeromonas hydrophila was isolated from pond water samples from Bumula, Bungoma South and Bungoma West Sub Counties. In Bumula Sub County, *Aeromonas hydrophila* was susceptible to amikacin, ceftazidime, ciprofloxacin, cefotaxime, cefepime, gentamicin and nalidixic acid with diameter of clear zones ranging from 21mm to 38mm (Plate 7). However, the bacterium was found to be moderately resistant to ampicillin and cefuroxime with diameter ranges of 5mm and 11mm respectively. In Bungoma South, the isolated *Aeromonas hydrophila* (Plate 8) was found to be resistant to ampicillin with diameter of 0mm but moderately susceptible to ceftazidime and cefuroxime at 24mm range of diameters each. Further, the bacterium was susceptible to amikacin, ciprofloxacin, cefotaxime, cefepime, gentamicin and nalidixic acid with diameter ranges from 20mm to 28mm. On the other hand, in Bungoma West, *Aeromonas hydrophila* (Plate 9) was found resistant to ampicillin at 0mm diameter and moderately susceptible to cefuroxime with a diameter of 20mm. The isolates were susceptible to amikacin, ceftazidime, ciprofloxacin, cefotaxime, cefepime, gentamicin and nalidixic acid with diameter ranges of 20mm to 34mm. *Aeromonas hydrophila* was isolated from pond water samples from Bumula, Bungoma South and Bungoma West Sub Counties (Figure 4). The tested antibacterial agents were found to be significantly different (P=0.00). Nevertheless, among the sub counties, the antibacterial agents were not significantly different (P=0.357).

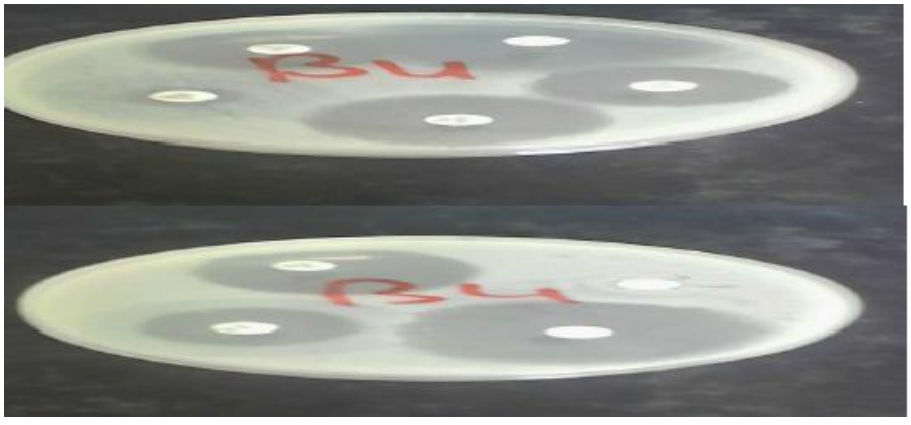

Plate 7. Impregnated sensitivity test for *Aeromonas hydrophila* as isolated from pond water in Bumula Sub County

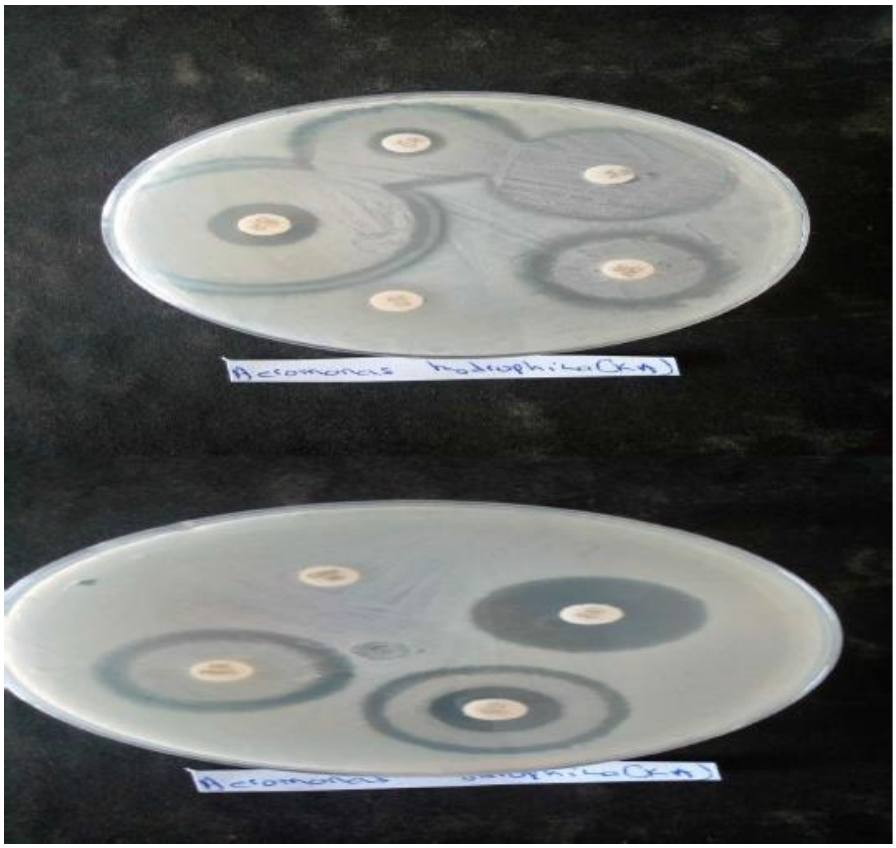

Plate 8. Impregnated sensitivity test for *Aeromonas hydrophila* as isolated from pond water in Bungoma South Sub County

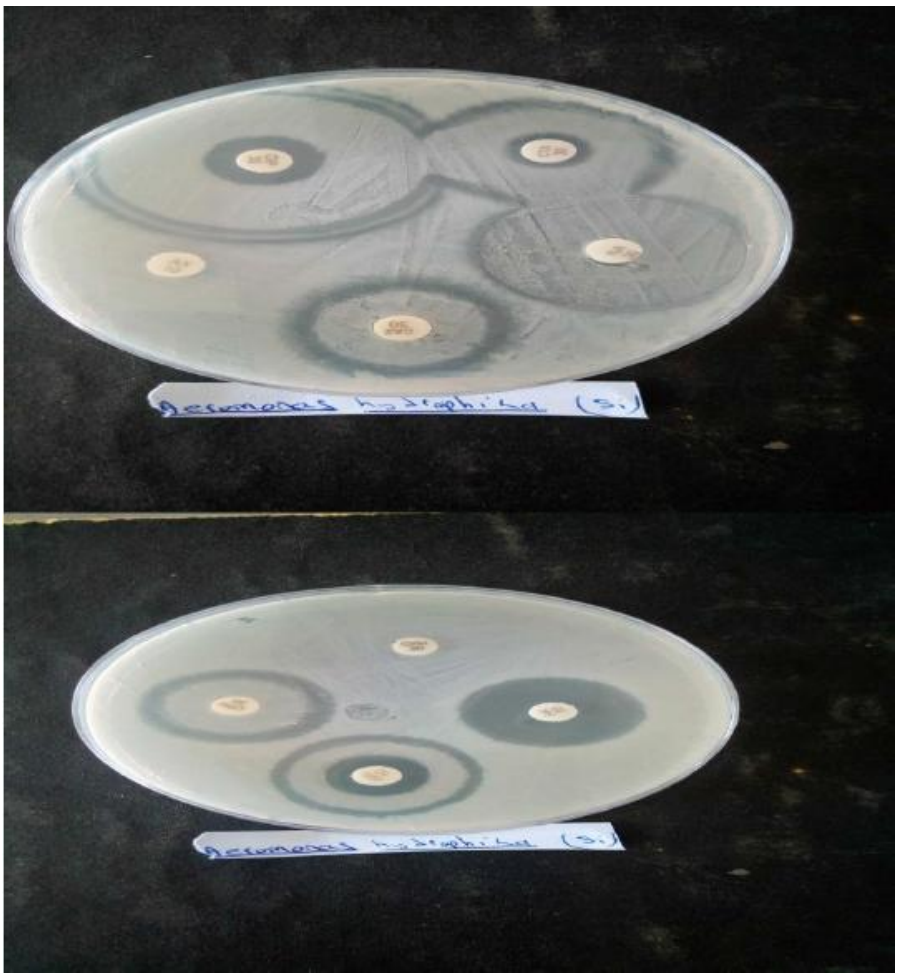

Plate 9. Impregnated sensitivity test for *Aeromonas hydrophila* isolated from pond water in Bungoma West Sub County

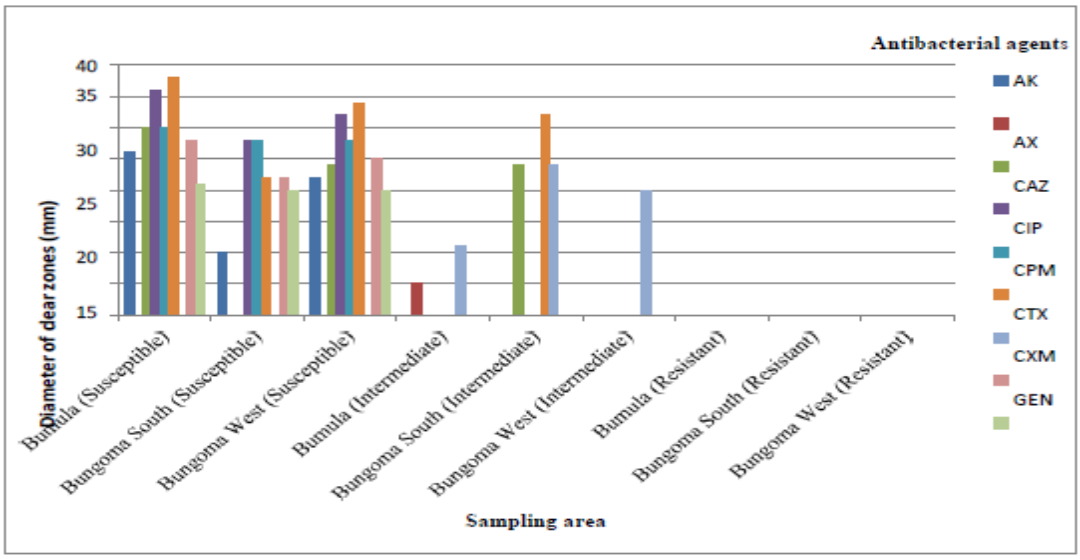

Figure 4. Sensitivity for bacterial isolates from pond water (AK-Amikacin, AX-Ampicillin, CAZ-Ceftazidime, CIP-Ciprofloxacin, CXM-Cefuroxime, CTX- Cefotaxime, CPM-Cefepime, GEN-Gentamicin, and NA-Nalidixic acid)

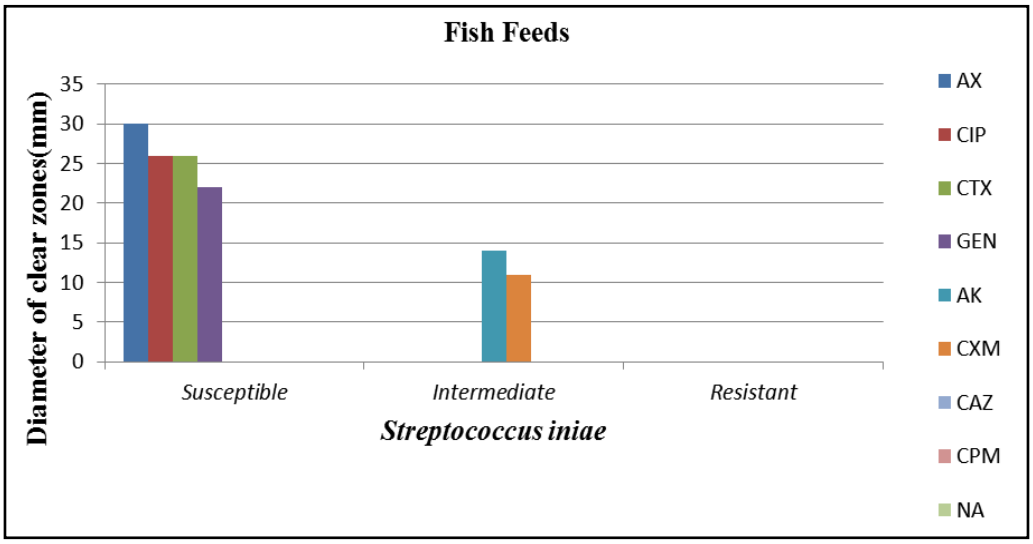

Figure 5. Responses of bacteria isolated from fish feeds (AK-Amikacin, AX-Ampicillin, CAZ-Ceftazidime, CIP-Ciprofloxacin, CXM-Cefuroxime, CTX- Cefotaxime, CPM-Cefepime, GEN-Gentamicin, and NA-Nalidixic acid)

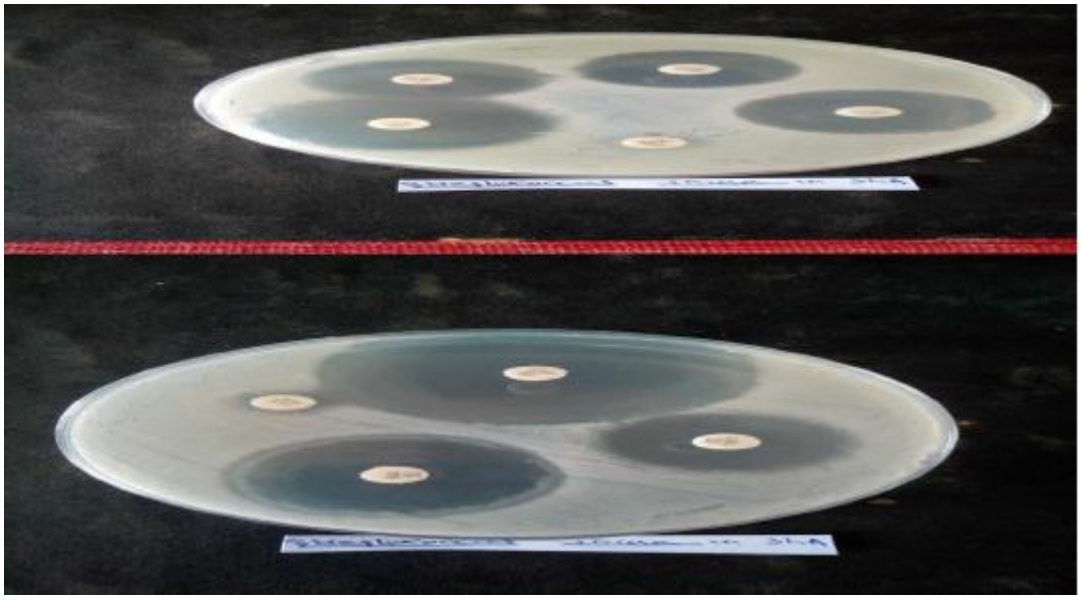

Plate 10. Impregnated sensitivity test for *Streptococcus iniae* isolated from fish feeds in Bungoma West Sub County fish feed miller

4.3. Responses of Pathogenic Bacteria Present in Fish Feeds

Streptococcus iniae isolated from fish feeds sourced from Bungoma West Sub County had varying sensitivity levels towards the tested antibacterial agents (Figure 5). It was found to be resistant to ceftazidime, ciprofloxacin and nalidixic acid with diameter ranges of 0mm and moderately susceptible to amikacin and cefuroxime at a diameter range of 14mm and 11mm, respectively. The recovered bacterium was susceptible to ampicillin, cefotaxime, cefepime and gentamicin with ranges from 20mm to 30mm (Plate 10).

4.4. Molecular Analysis

The $bla_{TEM\text{-}1a}$ gene was detected in all the five different bacterial strains. The $bla_{TEM\text{-}1a}$ gene was amplified about 424bp (Figure 6). Further, nucleotide sequences showed 100% sequences identity with the blaTEM-1a gene (GenBank Accession Numbers: BankIt2236899 Seq1a MN114035, BankIt2236899 Seq1b MN114036, BankIt2236899 Seq2a MN114037, BankIt2236899 Seq2b MN114038, BankIt2236899 Seq3a MN114039, BankIt2236899 Seq3b MN114040, BankIt2236899 Seq4a MN114041, BankIt2236899 Seq4b MN114042, BankIt2236899 Seq5a MN114043, BankIt2236899 Seq5b MN114044, BankIt2236899 Seq6a MN114045, BankIt2236899 Seq6b MN114046, BankIt2236899 Seq7a MN114047, BankIt2236899 Seq7b MN114048, BankIt2236899 Seq8a MN114049, BankIt2236899 Seq8b MN114050, BankIt2236899 Seq9a MN114051, and BankIt2236899 Seq9b MN114052).

The tree with the highest log likelihood (-560.5035) is shown in Figure 7. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 25 nucleotide sequences. Codon positions included were 1st+2nd+3rd+ Noncoding. There were a total of 373 positions in the final dataset.

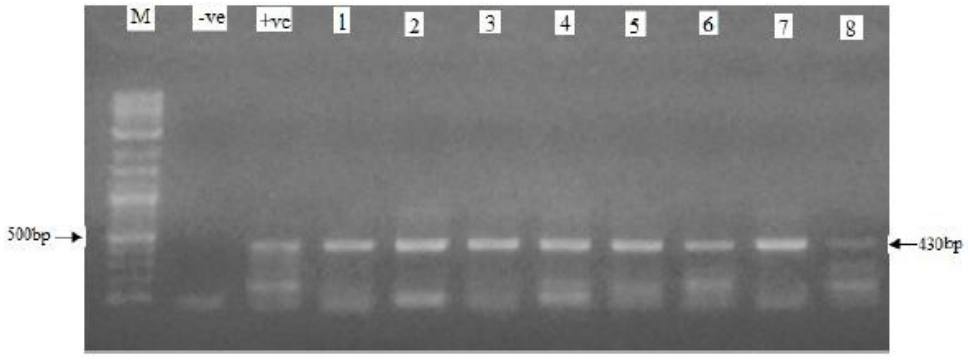

Figure 6. Molecular detection of presence of $bla_{TEM\text{-}1a}$; M; 100bp ladder, Negative control, Positive control-*Escherichia coli*, lane 1 *Vibrio parahaemolyticus* isolated from gills in Bungoma North; lane 2 *Vibrio vulnificus* isolated from fish scales in Bungoma South; lane 3 *Aeromonas hydrophila* isolated from pond water in Bungoma South; lane 4 *Pseudomonas aeruginosa* isolated from fish intestines in Bungoma West; lane 5 *Streptococcus iniae* isolated from fish feeds in Bungoma West; lane 6 *Aeromonas hydrophila* isolated from gills in Bungoma East; lane 7 *Aeromonas hydrophila* isolated from gills in Bungoma South; and lane 8 *Vibrio vulnificus* isolated from fish skin in Bungoma West

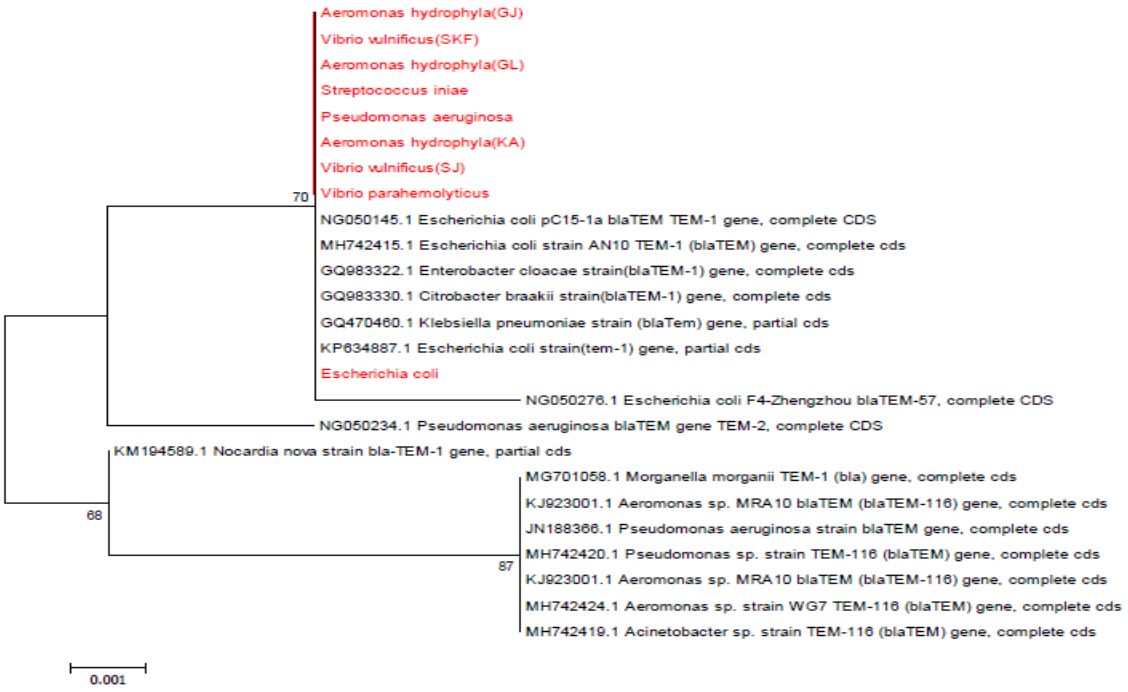

Figure 7. Phylogenetic tree of the extracted bacterial strains together with other strains with $bla_{TEM\text{-}1a}$ gene indicating similarities to other strains

5. Discussion

5.1. Sensitivity Tests

It was observed that *Vibrio vulnificus*, *Vibrio parahaemolyticus*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Streptococcus iniae* were susceptible, intermediate or resistant to tested antibacterial agents. *Vibrio vulnificus* from scales and skin was resistant to ampicillin but susceptible to ceftazidime, ciprofloxacin, cefotaxime and gentamicin. This implies that the bacterium possess ampicillin resistant genes within its chromosomes or plasmids. However, *Vibrio vulnificus* isolated from oysters of Louisiana Gulf in USA were susceptible to ciprofloxacin, gentamicin, cefotaxime and ceftazidime as well as ampicillin [15]. Further, the current study did not recover *Vibrio vulnificus* from pond water but *Vibrio vulnificus* isolates from German coastal waters were found susceptible to nalidixic acid, ampicillin, cefotaxime and ceftazidime while resistant to amikacin and gentamicin [16]. This indicates that *Vibrio vulnificus* in Louisiana Gulf and German coastal waters had not acquired ampicillin resistant genes.

In the current study, *Vibrio parahaemolyticus* was found to be susceptible to cefotaxime and ceftazidime. However, the bacterium isolated from Korean seafood was observed to be resistant to cefotaxime and ceftazidime [17]. It could be that the *Vibrio parahaemolyticus* isolated from fish in Bungoma County aquaculture systems had not developed any antibiotic resistance mechanisms. Further, *Vibrio parahaemolyticus* has been reported to be resistant to ampicillin, amikacin, cefotaxime and ceftazidime for isolates recovered from shellfish in Selangor, Malaysia [18]. It could be that *Vibrio parahaemolyticus* in marine environments had developed resistant genes against ampicillin, ceftazidime and cefotaxime unlike in freshwater aquaculture environment, where *Vibrio parahaemolyticus* has not acquired resistance genes. This is because sea and oceanic environments have other pollutants which force the fish to develop resistance. Such pollutants may not be in pond water.

Aeromonas hydrophila recovered from Nile tilapia were susceptible to amikacin, cefepime, ciprofloxacin, gentamicin, ceftazidime, cefuroxime and nalidixic acid. In agreement with the current study, *Aeromonas hydrophila* recovered from fish and crabs in Western Australia were susceptible to amikacin, cefepime, ciprofloxacin, gentamicin, ceftazidime and nalidixic acid [19]. This implies that the isolated *Aeromonas hydrophila* strains in the current study and those from Western Australia have not developed resistance against the aforementioned antibacterial agents. At the same time the *Aeromonas hydrophila* isolated in Bungoma East and Bungoma South sub counties were moderately susceptible to ciprofloxacin, cefotaxime as well as gentamicin and cefotaxime, cefuroxime and nalidixic acid respectively. However, *Aeromonas hydrophila* isolated from Nile tilapia from Al-manzala fish farms in Dakahlia governorate, Egypt found moderately susceptible to cefotaxime [38]. This implies that the strain could be possessing geographically different genes that confer mild resistance against antibacterial agents selected in this study. Furthermore, *Aeromonas hydrophila* from gills for samples from Bungoma South and Bungoma East Sub Counties were resistant to ampicillin in the present study. This concurs with the earlier study that reported *Aeromonas hydrophila* recovered from farmed *Tilapia mossambicus* in Malaysia being resistant to ampicillin [39,40].

Pseudomonas aeruginosa was found resistant to ampicillin and cefuroxime. Uniquely, the isolates had resistance radia at 3 mm against ampicillin whose cutoff is 13mm unlike other isolated bacterial pathogens in this study that had radia at 0mm against ampicillin. This implies that *Pseudomonas aeruginosa* is sparingly resistant to ampicillin compared to other recovered bacterial pathogens. On the other hand, *Pseudomonas aeruginosa* isolated from Armenian fish farms has concurrently been found resistant to ampicillin. This study also found that *Pseudomonas aeruginosa* was resistant to ampicillin indicating similarity in the ampicillin resistant genes [12]. Furthermore, it was observed during this study that among the bacterial isolates, only *Pseudomonas aeruginosa* was resistant to cefuroxime while the rest were susceptible. It maybe that *Pseudomonas aeruginosa* possesses cefuroxime resistant genes.

Aeromonas hydrophila isolated from water in the current study were susceptible to ciprofloxacin, gentamicin, ceftazidime, amikacin, cefepime, cefotaxime, cefuroxime and nalidixic acid but resistant to ampicillin. This could mean that the bacterium has developed tolerance against ampicillin over time being an environmental bacterium. Locally the bacterium has been recovered from River Njoro in Nakuru County (wild aquatic environment) and found to be resistant to ampicillin, gentamicin and ceftazoxime but sensitive to ciprofloxacin, nalidixic acid ceftazidime and cefotaxime [22]. Besides, *Aeromonas hydrophila* isolated from wastewater in Eastern Cape Province, South Africa were resistant to ampicillin but with susceptibility against gentamicin, cefotaxime, ciprofloxacin and nalidixic acid [21]. It is noted that *Aeromonas hydrophila* isolated from sea cucumber, bivalves and sea sediments in Melaka in Malaysia were resistant to ampicillin and nalidixic acid [20]. This implies the bacterium uses different parameters such as inactivating drugs with enzymes and efflux pumps [24,25,26].

Streptococcus iniae recovered from fish feeds in this study were resistant to ceftazidime, cefepime and nalidixic acid but were intermediate to amikacin and cefuroxime. The key observation here is that the strain was susceptible to ampicillin unlike the other four bacterial pathogens isolated from Nile tilapia and pond water that were all resistant to ampicillin. This could be because *Streptococcus iniae* is Gram positive while the other four strains are Gram negative. *Streptococcus iniae* obtained from fish farms in Jeju Island, Korea were documented as being susceptible to cefotaxime [21]. Similarly, it was found in the current study that *Streptococcus iniae* was susceptible to cefotaxime implying that though the two strains are from different geographical areas they possess similar genes that are susceptible to cefotaxime.

5.2. Molecular Analysis

The recovery of bla_{TEM} gene in the five bacterial pathogens demonstrates that antibacterial resistance was

due to its presence. Further, the bla_{TEM} gene was isolated from plasmids meaning antibacterial resistance was plasmid-mediated. This observation concurs with other studies that had shown presence of bla_{TEM-1a} gene in *Aeromonas hydrophila*. The bla_{TEM-1a} gene was present in *Aeromonas hydrophila* recovered from River Njoro, Nakuru in Kenya [20] and in *Aeromonas hydrophila* isolated from wastewater samples in South Africa [19]. The bla_{TEM-1a} gene was also present in *Aeromonas hydrophila* isolated from wild water in Brazil [41]. However, another study disagrees with this study as it had observed that bacterial pathogens including *Aeromonas hydrophila* resistance against antimicrobials is chromosomally mediated [42].

In the current study, it was found that *Streptococcus iniae* was phenotypically resistant to ciprofloxacin, ceftazidime and nalidixic acid. However, it has been reported that *Aeromonas* spp. resistances against ciprofloxacin and nalidixic acid is due to mutations in the *gyrA* region of the QRDR [43,44]. The recovery of bla_{TEM} gene from *Pseudomonas aeruginosa* in the current study is in concurrence with earlier reports [45]. Previous studies have shown that predominant ESBL genes in *Pseudomonas aeruginosa* are TEM (Temoneira), SHV (Sulfhydryl), CTX-M (Cefotaximase), PER (*Pseudomonas* ESBL), VEB (Vietnamase ESBL) and GES (Guiana ESBL) types from different parts of the World [45].

6. Conclusion

The five pathogenic bacteria recovered in aquaculture systems in Bungoma County had varying sensitivity towards tested antibacterial agents. *Vibrio vulnificus* was susceptible to all tested agents except ampicillin. However, *Vibrio parahaemolyticus*, *Aeromonas hydrophila* and *Pseudomonas aeruginosa* recovered from Nile tilapia were found to have intermediate and resistant sensitivity to more than one antibacterial agent. Further, *Aeromonas hydrophila* recovered from pond water was resistant to more than one agent tested. Again, *Streptococcus iniae* isolated from fish feeds was resistant to more than three agents. Therefore, it is concluded that *Vibrio parahaemolyticus*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Streptococcus iniae* are multi-drug resistant bacterial strains unlike *Vibrio vulnificus* that was found to be single drug resistant.

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