High Proportions of Multidrug-Resistant *Escherichia coli*, *Klebsiella pneumoniae*, and Other Gram-Negative Bacteria in Farmed Fish from Dar es Salaam, Tanzania

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ABSTRACT

One of the biggest problems the world is currently experiencing is the rapid rise of antimicrobial resistance, especially in developing nations. This cross-sectional study determined the antimicrobial susceptibility pattern of *Escherichia coli*, *Klebsiella pneumoniae*, and other Gram-negative bacteria isolated from farmed fish, manures, pond water, and fish feed in Dar es Salaam, Tanzania. 210 isolates belonging to 17 bacteria species, most predominantly *K. pneumoniae* (38.6%), followed by *E. coli* (17.1%) were recovered. Overall, the highest resistance was against ampicillin (80.5%), cefoxitin (29.5%), doxycycline (21.4%), and sulfamethoxazole-trimethoprim (15.2%). Of the 210 isolates, 68 (32.38%) were multidrug-resistant (MDR), with some isolates being resistant to 5 or more different classes of antibiotics, including cefoxitin, cefotaxime and ceftazidime (cephalosporins), imipenem, and meropenem (carbapenems), tetracyclines, ciprofloxacin (quinolones) and sulfamethoxazole (sulphonamide). The most frequent MDR patterns for *E. coli* were ampicillin-doxycycline-sulfamethoxazole-trimethoprim and ampilicilin-ciprofloxacin-doxycycline-sulfamethoxazole-trimethoprim, each was observed in 18.8% of the isolates. The highest proportion of MDR bacteria was found in manure (64.3%), followed by water (31.0%), fish (30.2%), and feeds (20.0%). The abundance of resistant bacteria in the aquaculture environment is concerning since they can be transferred across human-animal-environmental compartments, thereby affecting the whole ecosystem.

INTRODUCTION

In Tanzania, aquaculture production is a critical option to bridge the demand gap of more than 350,000 metric tons of fish and to raise the national per capita fish

consumption from 7.6 to 20.3kg **(URT, 2020)**. In this regard, the country has started to implement several initiatives to promote aquaculture development. Reports show that the number of earthen fish ponds has increased from 18,200 in 2010, producing 952 tonnes per year, to 21,300 fishponds in 2015, producing 31,1800 tonnes per year **(Rukanda, 2018)**. In 2012, according to the World Bank collection of development indicators compiled from officially recognized sources, the aquaculture production in Tanzania was reported at 10,6482 metric tons **(URT, 2020)**.

With the growing trend of aquaculture production in Tanzania, the use of antimicrobial agents intended for livestock in fish farming is likely to occur **(Mdegela *et al.*, 2021)**. This is happening at a time when the use of antimicrobial agents in livestock production is forecasted to increase by 67% from 2017 to 2030 **(Tiseo *et al.*, 2020)**. Sulphonamides, tetracycline, and quinolones are the most common antibiotics used by farmers for growth promotion, prophylaxis, and treatment purposes **(Kimera *et al.*, 2020; Mshana *et al.*, 2021; Ulomi *et al.*, 2022)**. In addition, there is an indirect introduction of antimicrobial agents in the aquaculture environment through integrated aquaculture-agriculture (IAA) practices that include fertilization of fish ponds using treated animal manure from swine, poultry, and cattle **(Shah *et al.* 2012; Minich *et al.*, 2018)**.

There are major concerns surrounding the use of antimicrobial agents in farm-raised fish, including the development of antimicrobial-resistant pathogens and antimicrobial residues in fish and the aquatic environment **(Cabello *et al.*, 2016)**. Unfortunately, the use of a specific antibiotic has been shown to promote resistance against other classes of antibiotics **(Zeng *et al.*, 2019)**.

This could affect the microbiota and resistomes of adjacent environments and beyond, suggesting an urgent need for surveillance of AMR in aquaculture **(Preena *et al.*, 2020)**.

Gram-negative bacteria such as *Escherichia coli* and *Klebsiella pneumoniae* are used in many laboratory-based surveillance of AMR, as these organisms are ubiquitously distributed in nature, food, animals, and humans and reflect AMR characteristics arising from selective pressure across these environments **(Anjum *et al.*, 2021)**. The World Health Organization (WHO) has classified *E. coli* and *K. pneumoniae* as critical priority pathogens due to their resistance to multiple antibiotics, such as carbapenems and third-generation cephalosporins, which are the most effective antibiotics for treating bacteria that are resistant to multiple drugs **(WHO, 2024)**.

In Africa, there is a scarcity of data in most parts of the continent since most studies have been conducted in South Africa, Egypt, Algeria, and Nigeria **(Brahmi *et al.*, 2018; Faleye *et al.*, 2018; Okon *et al.*, 2022)**. In realizing this, the second Tanzanian national action plan on the antimicrobial resistance of 2023-2028 has strongly emphasized the need to focus on AMR surveillance in the aquaculture sector in the country **(URT, 2022)**.

We carried out this study in Tanzania, aiming to create awareness and understanding of antimicrobial resistance in aquaculture to stakeholders (government

agencies, private-sector aquaculture producers, and aquatic animal health professionals). Providing such information is vital in creating awareness necessary for optimizing the use of antimicrobial agents in the sector, thereby reducing the AMR burden while maintaining the efficacy of antimicrobial agents. This is in line with the objectives of the Tanzanian National Action Plan on antimicrobial resistance (NAP-AMR) 2023-2028 (URT, 2022), the Africa Union Framework on AMR 2020 – 2025 **(AU, 2020)**, the 2021 Joint Tripartite (FAO, OIE, WHO) and UN Environment Programme (UNEP) initiative, which jointly aims to sustainably balance and optimize the health of people, animals, and ecosystems **(FAO, UNEP WHO & WOAH, 2023)**.

MATERIALS AND METHODS

Study area and design

This was a cross-sectional study carried out between March and June 2023 in Dar es Salaam, the largest city and business center of Tanzania, which has the highest population of 5,383,728 (8.7% of all Tanzanians) and a population density of 3100 inhabitants per km^2 (2022 Population and Housing Census). Regarding livestock production, Dar es Salaam has the country's highest density of livestock and fish farms **(de Glanville *et al.*, 2020)**.

Selection of fish farmers

We obtained a list of 47 fish farmers in Dar es Salaam from the Aquaculture Department of the Ministry of Livestock and Fisheries, Dar es Salaam, and the Coast Zone Office. We enlisted the assistance of the District Fisheries Officers in identifying and recruiting the fish farmers. Using this approach, we managed to visit 60 fish farms.

Sample collection and transportation

A total of 189 samples from fish (n = 88), pond water (n = 48), fish feed (n = 38), and animal manure (n =15) were collected. The number of samples per farm depended on the number of active ponds at each farm, the type of fish feed used, and the use and availability of animal manure during the sampling period.

Fish were caught using a scoop net wrapped in sterile polythene bags, while water samples were collected using sterile 50mL bottles, whereas manure and fish feeds were collected using a sterile spatula and placed in polystyrene plastic bags. Water and fish samples were transported in a cool box of 2- 8°C. In contrast, manure and feed samples were transported in a dry cool box to the Microbiology Teaching Laboratory at the Muhimbili University of Health and Allied Sciences (MUHAS). Upon arrival, samples were processed within 3 hours of collection.

Sampling process

In the laboratory, samples were processed as follows: i) for fish, sterile cotton wool was used to swab the gills and flesh muscle tissue; ii) 10g of feed and manure was diluted in sterile distilled water at a ratio of 1:10.

Bacterial isolation and identification

Processed samples were inoculated on MacConkey agar (Oxoid, Basingstoke, UK) and incubated at 37°C for 24 hours. Thereafter, bacterial growth was examined for Gram reaction as well as colony morphology and the ability of the bacteria to ferment lactose in MacConkey agar (Oxoid, Basingstoke, UK). Based on their lactose fermenting ability, bacteria were classified as lactose fermenters and non-lactose fermenters **(Mazumder *et al.*, 2022)**. Thereafter, single pure colonies were subcultured on MacConkey agar and incubated at 37°C for 24h for further biochemical identification, including oxidase, Kliger's Iron Agar (KIA), citrate, sulfur-indole-motility (SIM) and urease tests **(Darwich *et al.*, 2019)**. The VITEK 2 (bioMérieux, Inc. Hazelwood, MO, USA), which is an automated microbiology system utilizing growth-based technology, was used to identify bacteria that could not be identified by conventional biochemical tests. The VITEK 2 system uses colorimetric reagent cards that are incubated and interpreted automatically. The reagent cards have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities, such as acidification, alkalinization, enzyme hydrolysis, and growth, in the presence of inhibitory substances. An optically clear film present on both sides of the card allows for the appropriate level of oxygen transmission while maintaining a sealed vessel that prevents contact with the organism-substrate admixtures.

Phenotypic antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method **(Bauer *et al.*, 1966)** on Mueller Hinton agar (Becton, Dickinson and Company, New Jersey, USA). The antibiotics tested were sulfamethoxazole-trimethoprim (23.75/1.25µg), doxycycline (30µg), ampicillin (10µg), cefoxitin (30µg), cefotaxime (30µg), gentamycin (10µg), ceftazidime (30µg), imipenem (10µg), meropenem (10µg) and ciprofloxacin (5µg) (Oxoid Ltd, Basingstoke, Hampshire, United Kingdom). Colonies from pure cultures were emulsified into 5mL of sterile saline and adjusted to match the turbidity standard of 0.5 McFarland units (prepared by mixing 0.5mL of 1.75% (w/v) barium chloride dehydrate with 99.5mL of 1% (v/v) sulfuric acid). Emulsified broths were inoculated onto Mueller Hinton agar plates (Becton, Dickinson and Company, New Jersey, USA) using sterile cotton swabs and incubated at 37°C for 16 to 18h. The inhibition zone of each antimicrobial agent was measured using a ruler, and the results were interpreted according to CLSI standards 2023 (Clinical and Laboratory Standards Institute (CLSI), 2023). *E. coli* strain ATCC 29522 and *K. pneumoniae* strain

ATCC 700603 were used as controls. Multidrug-resistant isolates were defined as those that exhibited resistance to three or more different classes of antibiotics (**Magiorakos *et al.*, 2012**).

Statistical analysis

All data obtained during the study were recorded on a Microsoft® Excel spreadsheet. Recorded data included isolated bacteria, and phenotypic susceptibility profiles, including multidrug resistance. Descriptive statistics were performed under a 95% confidence interval, and SPSS was used to describe the frequency of occurrence of resistant bacteria and phenotypic antimicrobial susceptibility. Chi-square or Fisher's exact test was used for comparison between proportions when appropriate. Statistical significance (unadjusted *P*-value) was set at 0.05.

RESULTS

1. Bacteria isolates profile

A total of 210 isolates belonging to 17 bacteria species were recovered from the 189 processed samples. The most predominant species were *K. pneumoniae* (38.6%), followed by *E. coli* (17.1%), *Enterobacter* spp. (12.4%), *Aeromonas* spp. (9.5), and *Citrobacter* spp. (6.2%), collectively accounting for 83.8% of all isolates (Fig. 1).

As shown in Table (1), *K. pneumoniae* was the most predominant isolate in fish, water, and feed samples accounting for 41.6%, 33.3, and 60% of isolates, respectively, whereas *E. coli* was predominant in manure (57.1%). *Aeromonas* spp. were frequently found in water and fish samples. The rest of the isolates were found in small numbers.

2. Antimicrobial resistance profile of isolated bacteria

The overall antimicrobial-resistant pattern is shown in Fig. (2). Irrespective of sample source, resistance was highest against ampicillin (80.5%), followed by cefoxitin (29.5%), doxycycline (21.4%), and sulfamethoxazole-trimethoprim (15.2%). The least resistance was seen against ceftazidime (1.9%), followed by gentamycin (2.4%) and cefotaxime (7.6%). Table (2) shows that *K. pneumoniae* and *Aeromonas* spp. were highly resistant to ampicillin with percentage of 90.1 and 90%, respectively. *Citrobacter* and *Enterobacter* spp. were particularly resistant to Cefoxitin, with percentage resistance exceeding 80%.
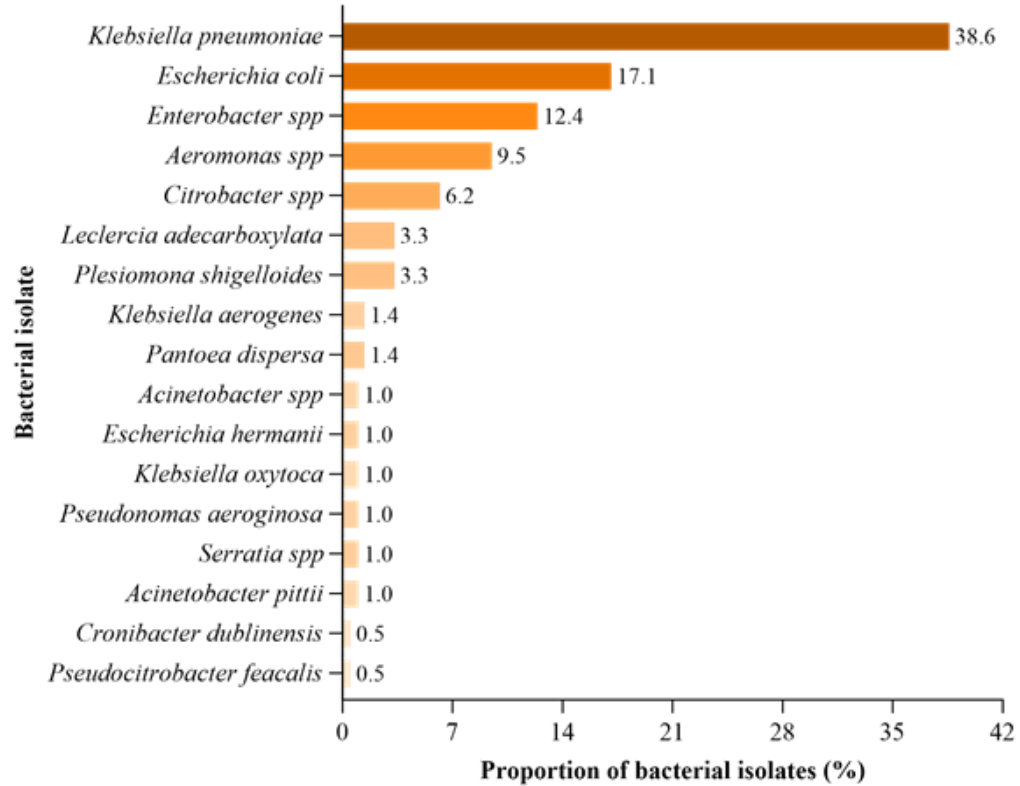
Fig. 1. The overall isolation frequency of bacteria isolated from all processed samples (n = 210)

Table 1. Isolation frequency of bacteria per sample source (n = 210)

| Bacterial isolates	Fish n = 149 n (%)	Water n = 42 n (%)	Feed n = 5 n (%)	Manure n = 14 n (%)
E. coli	20 (13.4)	8 (19.0)	0 (0.0)	8 (57.1)
K. pneumonia	62 (41.6)	14 (33.3)	3 (60.0)	2 (14.3)
Acinetobacter spp.	2 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)
Aeromonas spp.	11 (7.4)	8 (19.0)	0 (0.0)	1 (7.1)
Citrobacter spp.	9 (6.0)	3 (7.1)	1 (20.0)	0 (0.0)
C. dublinensis	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)
Enterobacter spp.	21 (14.1)	3 (7.1)	1 (20.0)	1 (7.1)
E. hermanii	2 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)
K. aerogenes	1 (0.7)	2 (4.8)	0 (0.0)	0 (0.0)
K. oxytoca	1 (0.7)	1 (2.4)	0 (0.0)	0 (0.0)
L. adecarboxylata	7 (4.7)	0 (0.0)	0 (0.0)	0 (0.0)
Pantoea dispersa	2 (1.3)	1 (2.4)	0 (0.0)	0 (0.0)
P. shigelloides	6 (4.0)	1 (2.4)	0 (0.0)	0 (0.0)
P. faecalis	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)
P. aeruginosa	1 (0.7)	0 (0.0)	0 (0.0)	1 (7.1)
Serratia spp.	0 (0.0)	1 (2.4)	0 (0.0)	1 (7.1)
Acinetobacter pittii	2 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)
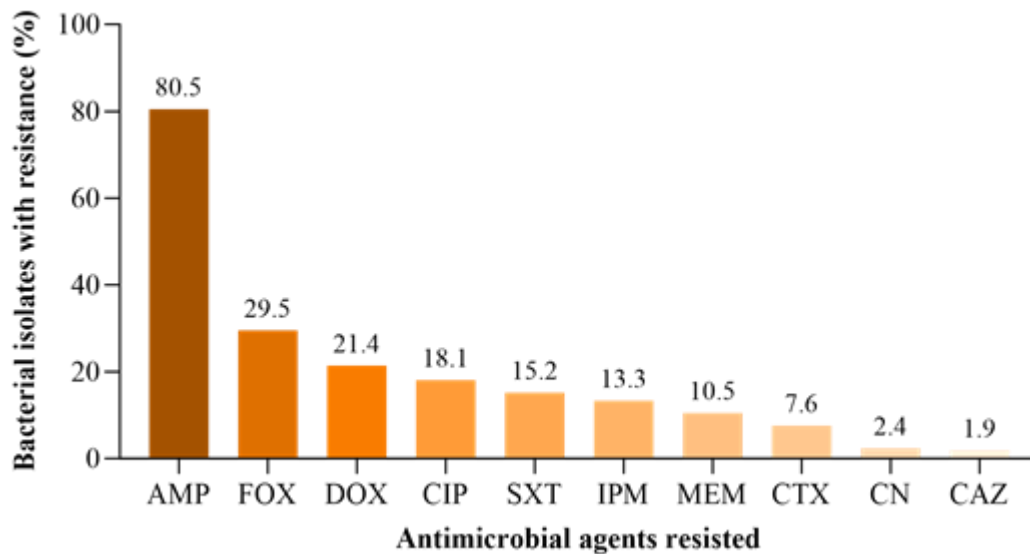
Fig. 2. The overall Antimicrobial resistance pattern (%) of all bacteria isolated from farmed fish and the surrounding aquaculture environment (n = 210)

Table 2. Antimicrobial resistance pattern of all bacteria isolated from farmed fish and the surrounding aquaculture environment (n = 210)

Bacterial species	N	Percentage resistance									
		AMP	CTX	CAZ	MEM	IPM	FOX	CN	CIP	DOX	SXT
Overall	**210**	**80.5**	**7.6**	**1.9**	**10.5**	**13.3**	**29.5**	**2.4**	**18.1**	**21.4**	**15.2%**
E. coli	36	58.3	13.9	2.8	5.6	13.9	5.6	8.3	27.8	41.7	27.8
K. pneumonia	81	90.1	0.0	0.0	7.4	2.5	8.6	0.0	17.3	19.8	13.6
Aeromonas spp.	20	90.0	0.0	0.0	15.0	50.0	30.0	0.0	15.0	0.0	5.0
Citrobacter spp.	13	76.9	0.0	0.0	23.1	7.7	84.6	0.0	30.8	0.0	0.0
Enterobacter spp.	26	84.6	15.4	3.8	15.4	34.6	96.2	3.8	15.4	42.3	15.4
Others	34	73.5	20.6	5.9	11.8	2.9	32.4	2.9	8.8	8.8	17.6

Key: AMP: Ampicillin, DOX: Doxycycline, SXT: Sulfamethoxazole-trimethoprim, FOX: Cefoxitin, CTX: Cefotaxime, CAZ: Ceftazidime, IMP: Imipenem, MEM: Meropenem, GN: Gentamycin, CIP: Ciprofloxacin

Isolates from manure samples had considerably higher resistance to trimethoprim/sulfamethoxazole and doxycycline, as shown in Fig (3). Other drugs show no significance from all sources of samples.
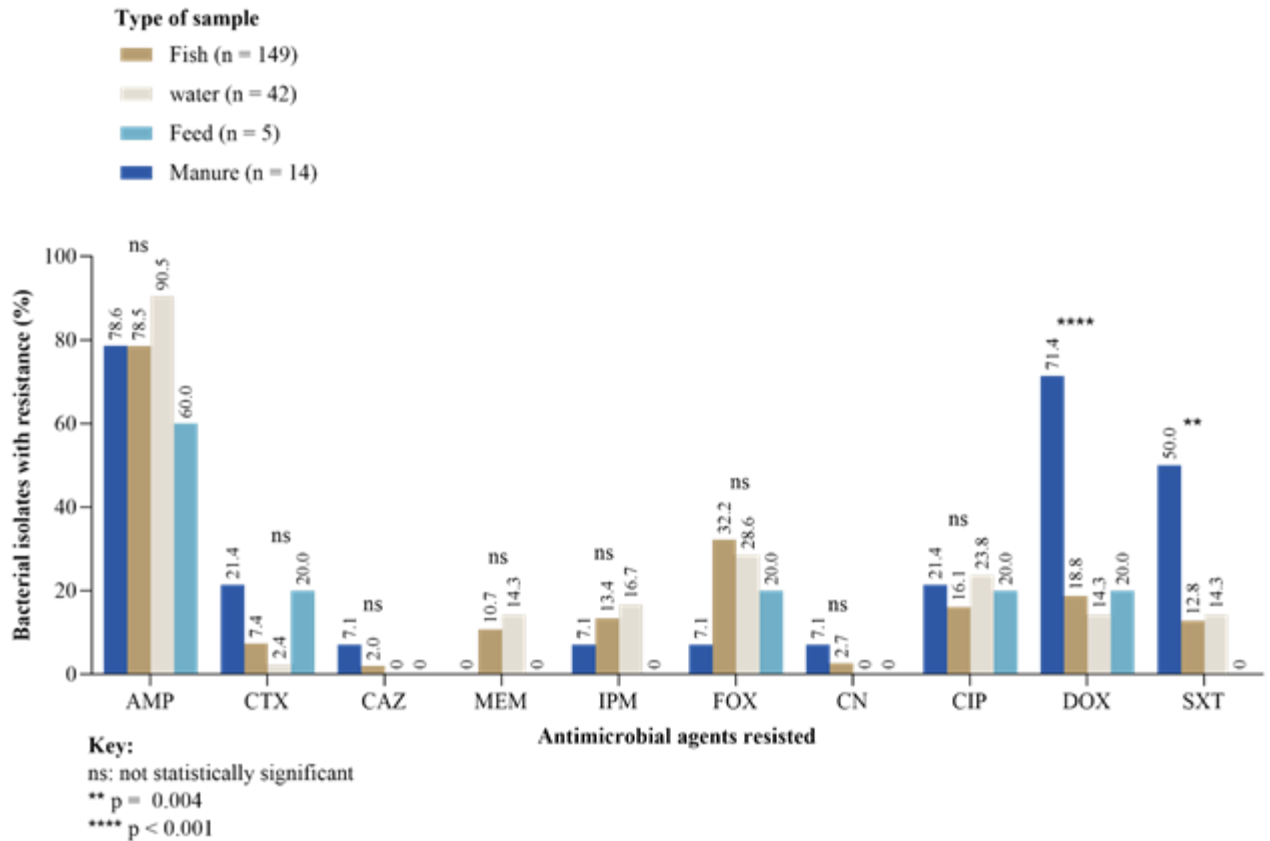
Fig. 3. Antimicrobial resistance pattern of isolate according to source of samples

The resistance patterns of *E. coli* and *K. pneumoniae* are compared in Fig. (4). The percentage resistance against ampicillin was higher for *K. pneumoniae* (90.1%) versus 58.3% for *E. coli*. *E. coli* was significantly more resistant against cefotaxime (13.9% vs 0%), ciprofloxacin (27.8% vs 17.3%), doxycycline (41.7% vs 19.8%) and sulfamethoxazole-trimethoprim (27.8% vs 13.6%).
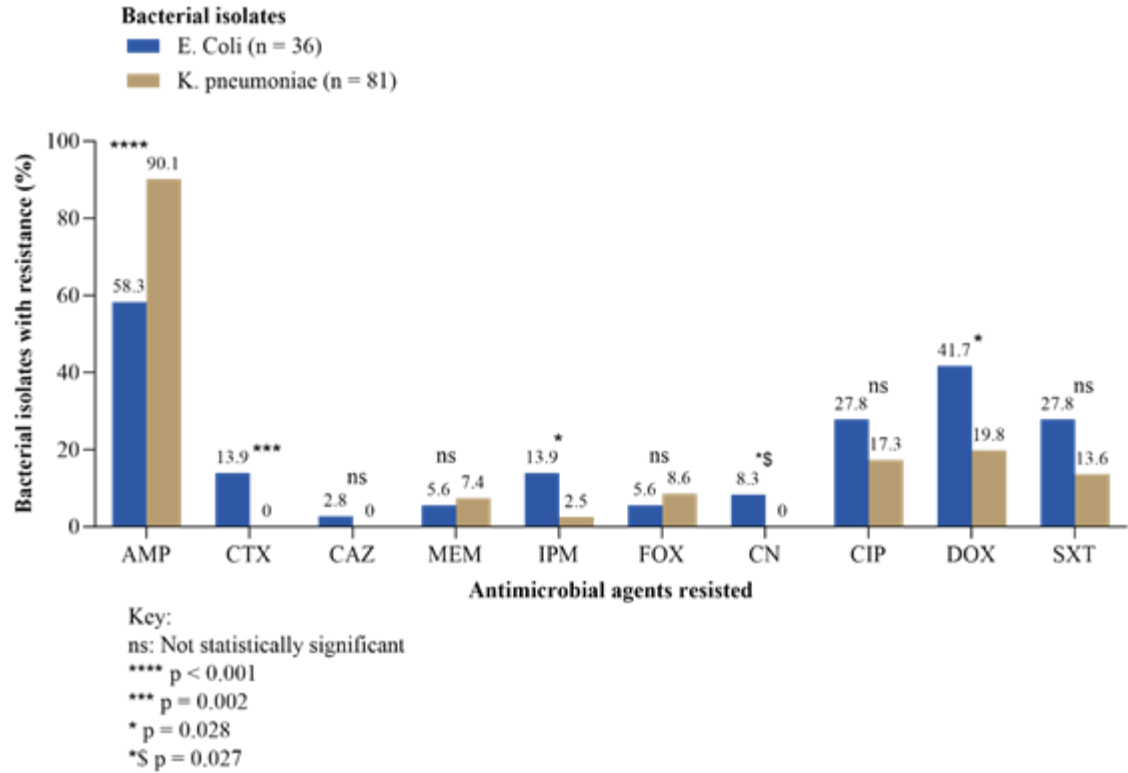
Fig. 4. The resistance pattern of *E. coli* and *K. pneumoniae*

3. Multidrug resistance pattern of bacteria isolates

Of the 210 isolates, 68 (32.38%) were multidrug-resistant (MDR), the most frequent combination being ampicillin, doxycycline, and sulfamethoxazole-trimethoprim (Table 3). The same table shows that some isolates were resistant to 5 or more different classes of antibiotics, including cefoxitin, cefotaxime, ceftazidime, imipenem, and meropenem.

Table 3. Overall antimicrobial resistance pattern of all MDR bacteria n = 68

Antibiotic combination	Number of isolates (n)	Percentage (%)	Number of antibiotic classes
AMP+ DOX+ SXT	8	11.8	3
AMP+ MEM+ CIP	4	5.9	3
AMP +CIP+ DOX	3	4.4	3
AMP+ FOX+ DOX	3	4.4	3
AMP +IPM+ FOX	3	4.4	3
AMP+ MEM+ FOX	2	2.9	3
AMP+ MEM +IPM	2	2.9	2
MEM+ IPM +FOX	1	1.5	2
AMP+ CAZ+ FOX	1	1.5	3
AMP +FOX +SXT	1	1.5	3
AMP+ CIP+ SXT	1	1.5	3
AMP+ CTX+ DOX	1	1.5	3
AMP+ CTX+ FOX	1	1.5	3
AMP +CTX +IPM	1	1.5	3
AMP +FOX+ CIP	1	1.5	3
CIP +DOX +SXT	1	1.5	3
AMP +CIP+ DOX +SXT	7	10.3	4
AMP +IPM+ FOX+ DOX	3	4.4	4
AMP+ FOX +DOX+ SXT	3	4.4	4
AMP +MEM+ IPM +FOX	3	4.4	3
AMP +MEM+ FOX +CIP	2	2.9	4
AMP +CN+ CIP+ DOX	2	2.9	4
AMP+ MEM+ IPM+ CIP	1	1.5	3
AMP+ CTX+ FOX +SXT	1	1.5	4
AMP+ CTX +IPM +CIP	1	1.5	4
AMP +CTX +IPM+ FOX	1	1.5	4
AMP+ FOX+ CIP +DOX +SXT	1	1.5	5
AMP +CTX +CAZ +DOX +SXT	1	1.5	4
AMP +CTX+ FOX+ CIP +DOX	1	1.5	5
AMP +CTX +FOX +DOX +SXT	1	1.5	5
AMP+ CTX +CAZ+ FOX +CIP+ SXT	1	1.5	5
AMP +CTX+MEM+IPM+FOX+CIP + DOX	1	1.5	6
AMP+CTX+MEM+IPM+CIP+DOX+SXT	1	1.5	6
CTX+CAZ+IPM+FOX+CN+CIP+DOX	1	1.5	6

Key: AMP: Ampicillin, DOX: Doxycycline, SXT: Sulfamethoxazole-trimethoprim, FOX: Cefoxitin, CTX: Cefotaxime, CAZ: Ceftazidime, IMP: Imipenem, MEM: Meropenem, CN: Gentamycin, CIP: Ciprofloxacin.

MDR *E. coli* isolates were resistant to 3 to 6 different classes of antibiotics. The most frequent MDR patterns for *E. coli* were AMP+DOX+SXT and AMP+CIP+DOX+SXT, each pattern accounts for 18.8% of the isolates. Most MDR patterns contained ampicillin,

doxycycline, and ciprofloxacin (Table 3). As shown in Fig. (5) below, the highest proportion of MDR bacteria was found in manure (64.3%), followed by fish (30.2%), water (31.0%), and feeds (20.0%).
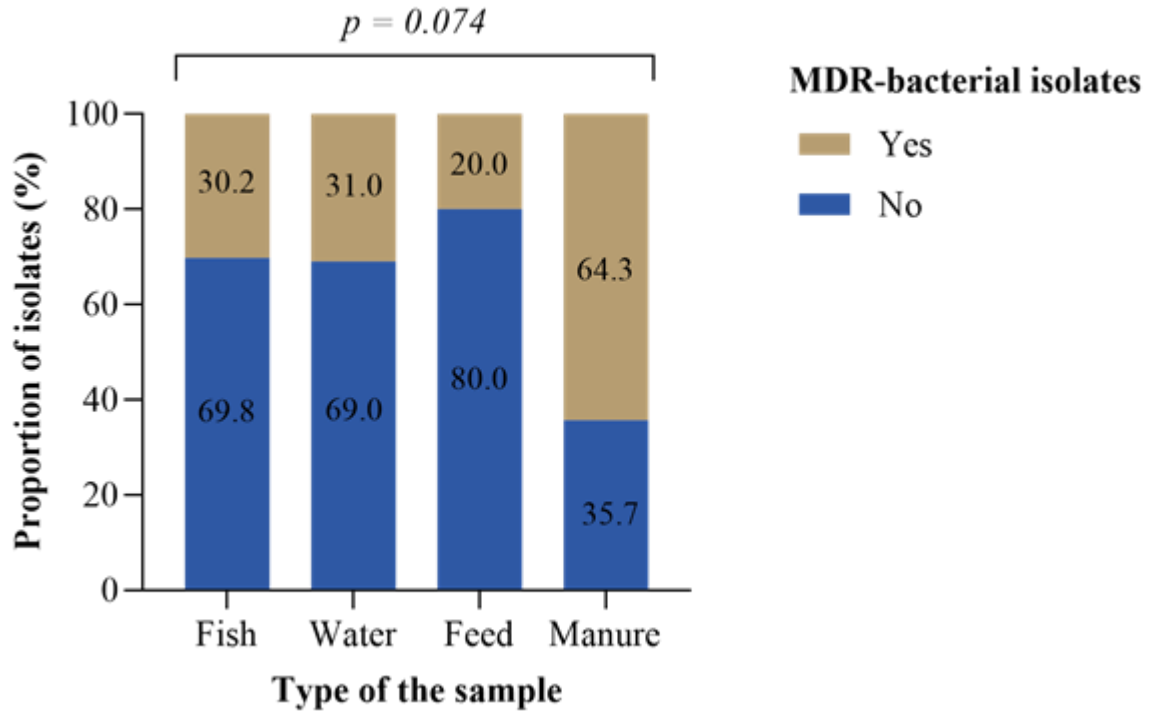

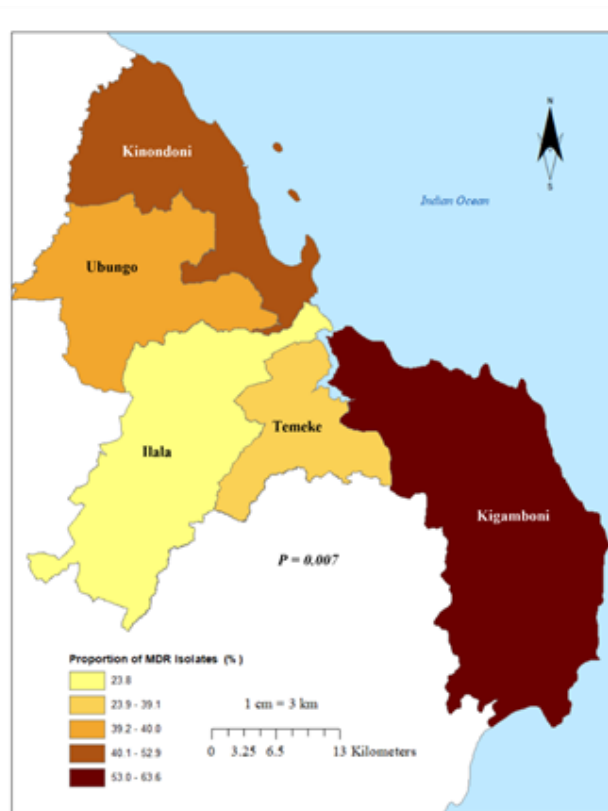**Fig. 5.** Distribution of MDR bacterial isolates per sample source

Fig. 6. Distribution of MDR bacterial isolates in the study area

As shown in Fig. (6), above areas close to the Indian Ocean had a significantly higher proportion of MDR isolates (53.0- 63.6%) and decreased to 39%, and then 23.8% moving away from it.

Table 4. Antimicrobial resistance pattern of multidrug-resistant *E. coli,* n = 16

Antibiotic combination	Number of isolates	Percentage	Number of antibiotic classes
AMP+CIP+DOX	1	6.3	3
AMP+CIP+DOX+SXT	3	18.8	4
AMP+CN+CIP+DOX	2	12.5	4
AMP+CTX+CAZ+DOX+SXT	1	6.3	4
AMP+CTX+ IPM	1	6.3	3
AMP+CTX+IPM+CIP	1	6.3	4
AMP+CTX+MEM+IPM+CIP+DOX+SXT	1	6.3	6
AMP+DOX+SXT	3	18.8	3
AMP+IPM+FOX	1	6.3	3
CIP+DOX+SXT	1	6.3	3
CTX+DOX+SXT	1	6.3	3

Key: AMP: Ampicillin, DOX: Doxycycline, SXT: Sulfamethoxazole-trimethoprim, FOX: Cefoxitin, CTX: Cefotaxime, CAZ: Ceftazidime, IMP: Imipenem, MEM: Meropenem, CN: Gentamycin, CIP: Ciprofloxacin.

Table 5. Antimicrobial resistance pattern of multidrug-resistant *K pneumoniae,* n = 16

Antibiotic combination	Number of isolates	Percentage	Number of antibiotic classes
AMP+CIP+DOX	2	12.5	3
AMP+CIP+DOX+SXT	4	25.0	4
AMP+DOX+SXT	4	25.0	3
AMP+FOX+DOX	1	6.3	3
AMP+FOX+DOX+SXT	1	6.3	4
AMP+MEM+CIP	3	18.8	3
AMP+MEM+IPM+CIP	1	6.3	3

Key: AMP: Ampicillin, DOX: Doxycycline, SXT: Sulfamethoxazole-trimethoprim, FOX: Cefoxitin, CTX: Cefotaxime, CAZ: Ceftazidime, IMP: Imipenem, MEM: Meropenem, CN: Gentamycin, CIP: Ciprofloxacin.

MDR *K. pneumnoniae* isolates were resistant to between 3 and 4 different classes of antibiotics. The most frequent MDR patterns for *K. pneumnoniae* were AMP+CIP+DOX+SXT and AMP+ DOX+SXT, each pattern accounts for 25% of the isolates. Resistance to ampicillin was seen in all MDR combinations, while resistance against doxycycline and ciprofloxacin was found in almost all combinations (Tables 4, 5).

DISCUSSION

In this study, we found a wide spectrum of bacteria, belonging to a total of 17 different species from the 277 processed samples. Among the 210 isolates that were recovered, *K. pneumoniae* (38.6%) was the most predominant, followed by *E. coli* (17.1%), *Enterobacter* spp. (12.4%), *Aeromonas* (9.5) and *Citrobacter* spp. (6.2%), collectively accounting for 83.8% of all isolates. However, we also isolated several relatively uncommon bacteria such as *Escherichia hermannii*, *Leclercia adecarboxylata*, *Pantoea dispersa*, *Acinetobacter pittii*, and *Pseudocitrobacter faecalis*. We found significant differences in isolation frequencies among the different sources of the sample. *K. pneumoniae* was the most predominant isolate in fish, water, and feed samples accounting for between 33 and 60% of all isolates, whereas *E. coli* was predominant in manure (57.1%). *Aeromonas* spp. were frequently found in water and fish samples. The rest of the isolates were found in small numbers.

In our study, most of the isolated bacteria were resistant to multiple antibiotics. Irrespective of sample source, resistance was at its highest against ampicillin (80.5%), followed by cefoxitin (29.5%), doxycycline (21.4%), and sulfamethoxazole-trimethoprim (15.2%). The least resistance was seen against ceftazidime (1.9%), followed by gentamycin (2.4%) and cefotaxime (7.6%). *Citrobacter* and *Enterobacter* spp. were particularly resistant to Cefoxitin, with percentage resistance exceeding 80%.

We found that resistance against ampicillin was higher for *K. pneumoniae* (90.1%) versus 58.3% for *E. coli*. On the other hand, *E. coli* was significantly more resistant than *K. pneumoniae* against cefotaxime (13.9 vs 0%), ciprofloxacin (27.8 vs 17.3%), doxycycline (41.7 vs 19.8%, and sulfamethoxazole-trimethoprim (27.8 vs 13.6%). Sixty-eight (32.38%) of the 210 isolates were resistant to more than three different classes of antibiotics and were classified as being MDR **(Magiorakos *et al.*, 2012)**. Resistance to ampicillin was seen in all MDR combinations, while resistance against doxycycline and ciprofloxacin was found in almost all combinations. Similar variations in resistance between *E. coli* (44.5%) and *K. pneumoniae* (27.3%) have been observed in wastewater and sewage within the same community, suggesting that enriched ARGs in aquaculture can impact nearby environments through horizontal gene transfer **(Kim & Cha, 2021; Seguni *et al.*, 2023)**. Our findings align with findings from Ghana, where isolates showed high resistance (≥70%) to penicillin, ampicillin, flucloxacillin, and tetracycline, with 44-92.9% exhibiting resistance to more than three antibiotics **(Agoba *et al.*, 2017)**.

Similarly, **Adekanmbi *et al.* (2021)** reported that 88.5% of bacteria from Nigerian aquaculture ponds were resistant to ampicillin. The AMR patterns seen among isolates in this study reflect common antibiotics used in livestock in Tanzania, which include tetracycline, sulphonamides, trimethoprim, quinolones, aminoglycosides, and beta-lactams **(Sangeda *et al.*, 2021)**.

On the other hand, the level of resistance seen in this study is lower than that reported in Uganda, which reported bacteria isolated from fish showed high (100%) resistance to penicillin, oxacillin, and ampicillin **(Wamala *et al.*, 2018)**, but significantly higher than that reported by **Adinortey *et al.* (2020)** in selected fish farms of the Central Region of Ghana, showing the proportion of resistant bacteria to be 28,6% for *Citrobacter freundii* (28.6%), 19.0% for *Klebsiella pneumoniae* (19.0%) and 17.5% for *Escherichia coli* (17.5%).

The variations in the level and pattern of antibiotic resistance between studies could be attributed to several reasons such as differences in the level of aquaculture management (intensive, semi-intensive, or extensive) and the extent of antibiotics use in fish farming, the sensitivity of analytical methods used and surveillance techniques (isolating, identifying, and testing bacteria susceptibility) **(Limbu *et al.*, 2023)**. We noted that comparing antimicrobial results of aquaculture studies conducted in Africa is rather difficult since these studies have used different pathogens and have tested them on different antimicrobial agents, hindering, to a large extent, comparison between countries **(Limbu *et al.*, 2023)**.

Our study involved a detailed technique (VITEK-2) that could identify pathogens that would have been missed by conventional biochemical tests. These findings seem to indicate manure is a major source of residual antibiotics, ARB, and diverse ARGs, and that its utilization in aquaculture leads to an increased abundance of resistant bacteria and resistance genes in the environment **(Xu *et al.*, 2022)**. We found significant geographical

variations in the proportion of MDR isolates, being higher in the areas close to the Indian Ocean (53.0- 63.6%), decreasing to 39%, and then 23.8% moving away from it. This may probably indicate variations in anthropogenic activities related to AMR (**Domínguez** *et al.,* **2021**). In conclusion, we emphasize that the findings of this study are important since they seem to indicate a large presence of AMR bacteria in the aquaculture environment, which can be transferred across human-animal-environmental compartments, thereby affecting the whole ecosystem through the flow of resistomes across One Health compartments (**Schar** *et al.***, 2020**).

Due to a lack of resources, this study did not incorporate molecular work such as Whole Genome Sequencing (WGS) and metagenomics, thus limiting the understanding of the flow of resistomes across the various compartments (manure, fish, and the aquatic environment). These techniques provide a complete genomic profile of isolates and can identify all genetic elements that may confer AMR, thus providing important information that can influence policies, develop preventative measures, and alleviate the burden caused by antibiotic resistance.

CONCLUSION

Our research shows a large spectrum of MDR Gram-negative bacteria in the aquaculture system, including resistance against carbapenem and third-generation cephalosporins like imipenem and meropenem. This is a major concern to public and animal health and the surrounding environment since AMR genes in aquaculture spread more efficiently than those in the terrestrial compartment. A comprehensive One Health integrated surveillance of AMR is recommended to monitor the passage of antimicrobial resistance genes and resistant bacteria from aquatic to terrestrial animal husbandry and humans and environment and vice versa to limit the detrimental effects to both human and animal health and on aquatic ecosystems.

Such an approach provides a more complete picture of AMR and AMU and facilitates analyses of trends over time and space and relations among the different compartments and between countries should use WHO, OIE, and the FAO/WHO guidelines and standards and systems for the collection and sharing of data on humans, food animals, plant production and environmental surveillance.

Based on the results of this study which show high levels of antimicrobial resistance and multi-dug resistance bacteria, and those of a previous study conducted in the same aquaculture ecosystem, showing high levels of antimicrobial residues (**Kilusungu** *et al.,* **2024**), we recommend a comprehensive and continuous surveillance of AMU and AMR in combating AMR and its associated adverse effects in aquaculture.

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