

BOOK OF ABSTRACTS

3rd International Conference on Sustainable Fisheries (ICSF) 2025

"Innovative solutions for resilient fisheries and aquaculture"

5-7 SEPTEMBER



Faculty of Fisheries
Sylhet Agricultural University
Sylhet-3100, Bangladesh
www.sau.ac.bd



CONTENTS

Pages

Conference Keynote	09-10
Thematic Area : Aquaculture and Nutrition	11-61
Thematic Area : Aquatic Animal Health and Pollution	62-103
Thematic Area : Aquatic Resource Management and Conservation	104-119
Thematic Area : Fisheries Biology, Genetics and Biotechnology	120-140
Thematic Area : Fishing, Post-harvest Technology, Seafood Safety and Quality	141-162
Thematic Area : Oceanography, Climate Change and Blue Economy	163-195
Thematic Area : Livelihood, Fisheries Policy and Governance	196-206
Poster Session	207-301

ANTIMICROBIAL RESISTANCE CHARACTERISTICS OF *Escherichia coli* ISOLATED FROM COMMERCIAL TILAPIA FARMS: A STUDY FROM A MAJOR FISH PRODUCTION HUB IN BANGLADESH

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In aquaculture, antimicrobials are commonly used to prevent and treat infections in fish; however, injudicious antimicrobial use (AMU) can pose significant risks, including the emergence of antimicrobial-resistant pathogens in aquatic environments. This study was carried out to assess the occurrence of drug-resistant *Escherichia coli* in tilapia (*Oreochromis niloticus*) collected from 41 commercial tilapia farms located in five upazilas (Fulbaria, Trishal, Phulpur, Tarakanda and Mymensingh Sadar) of Mymensingh, Bangladesh, between December 2022 and April 2023. A total of 153 samples, including skin (n=41), gill (n=41), intestine (n=30), and farm water (n=41) were analyzed. The *E. coli* isolates were isolated and then identified using the *E. coli*-specific *uidA* gene by means of polymerase chain reaction (PCR). Antimicrobial susceptibility testing (AST) was conducted by means of Kirby Bauer disk diffusion method with 14 antimicrobials to determine the antimicrobial resistance (AMR) patterns, and the results were interpreted by Clinical and Laboratory Standards Institute (CLSI) guidelines. In addition, the multiple antimicrobial resistance (MAR) index was calculated to determine the extent of multi-drug resistance in *E. coli*. A total of 31 *E. coli* isolates (20.26%) were identified, with the highest prevalence in the intestine (32.26%), followed by skin (29.03%), water (25.81%), and gill (12.90%). The isolates were mostly resistant to ampicillin (93.55%), nalidixic acid (38.71%), and oxytetracycline (32.26%). Conversely, more than 80% of the isolates were susceptible to chloramphenicol, meropenem, norfloxacin, gentamicin, azithromycin, levofloxacin, and cefepime. The MAR index values ranged from 0 – 0.71, with 38.71% of the isolates classified as multi-drug-resistant isolates, indicating selective pressure within the aquaculture environment. These findings highlight the possible risk of transmitting AMR bacteria through the aquatic food chain and emphasize the necessity for prudent AMU in aquaculture.



Antimicrobial Resistance Characteristics of *Escherichia coli* Isolated from Commercial Tilapia Farms: A Study from a Major Fish Production Hub in Bangladesh

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Presented by: Sabrina Hossain, PhD

WorldFish Bangladesh

September 05, 2025



Aquaculture in Bangladesh

- **Fifth-largest** inland aquaculture producer in the world
- Impressive average **annual growth** of **8.59%**
- Providing food and nutrition: 60% of animal food intake
- Livelihoods and employment: workplace for **17.8 million people** of which 7.8% are women
- Aquaculture is challenged by infectious diseases, which can lead to use of **antimicrobials**.



Antimicrobials and Antimicrobial Resistance (AMR)

Antimicrobial

- Synthetic, semi-synthetic or natural compound that has ability to **kill or inhibit** the growth of microbes

Antibiotic

- A chemical produced by microbes that has selective capacity to **inhibit growth or kill** other bacteria

“All antibiotics are antimicrobials but not the reverse”

Antimicrobial resistance (AMR)

- Ability of microbes to **resist the inhibition or killing effects** of antimicrobials, making the drugs less effective or ineffective

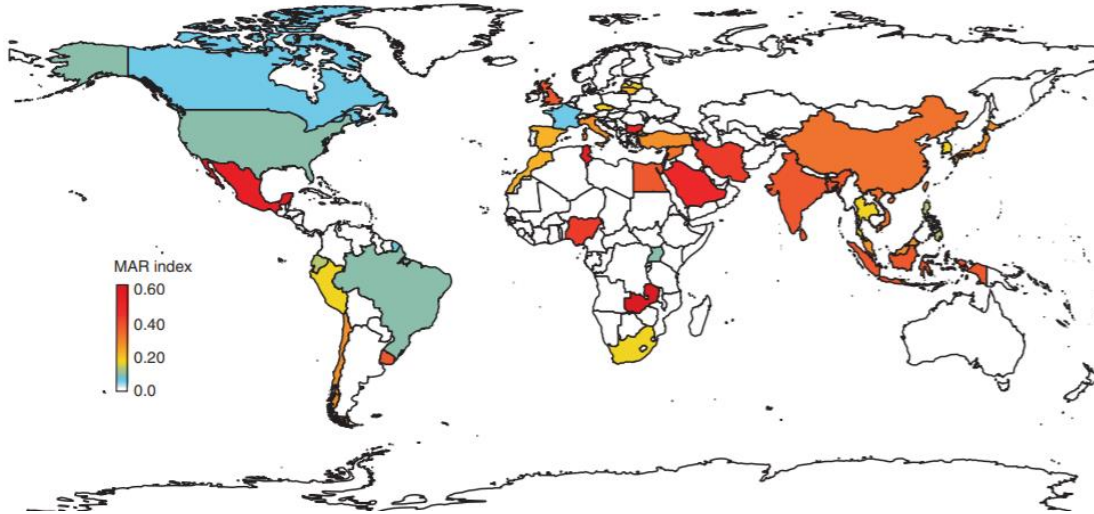
Pathways of AMR Transmission

- **Direct selection pressure:**
 - Antimicrobial use (AMU)
- **Gene transfer from the environment:**
 - Pond fertilization
 - Agricultural run off
 - Human effluent



Global Multiple Antimicrobial Resistance (MAR) index for Aquaculture

11,274 isolates from 40 countries



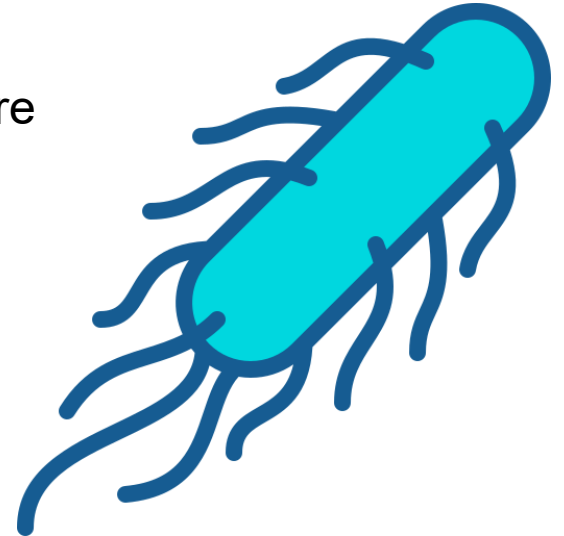
Reverter *et al.*, 2020.

<https://doi.org/10.1038/s41467-020-15735-6>

MAR indices > 0.2, considered as high-risk antimicrobial contamination

Why choosing *E. coli*?

- **Indicator of Fecal Contamination:** signals water and feed hygiene issues in fish/shrimp farms
- **Reservoir of AMR Genes:** can acquire and transfer resistance to other aquatic and pathogenic bacteria
- **Zoonotic & Public Health Concern:** links aquaculture to human health risks via food and environment
- **Easy & Cost-Effective for Surveillance:** simple isolation and standard use in [AMR surveillance](#) (OIE/WHO recommended)



Methodology

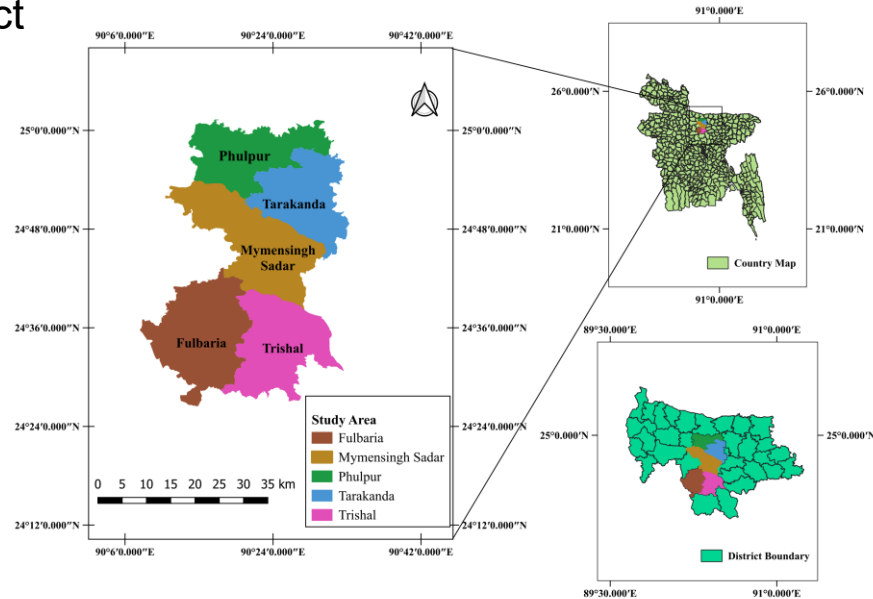
Study location: Five upazilas in Mymensingh district of Bangladesh

- Fulbaria
- Mymensingh Sadar
- Trishal
- Phulpur
- Tarakanda

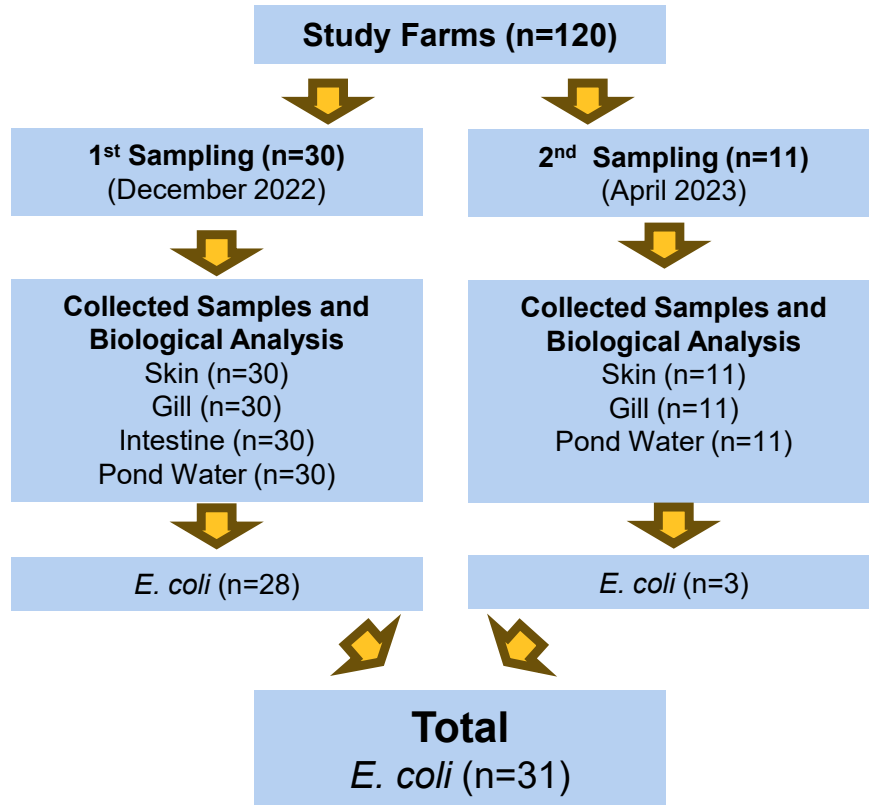
Sample collection: December 2022 and April 2023

Sample processing and laboratory analysis:

Bangladesh Livestock Research Institute (BLRI, Savar)



Sampling Overview



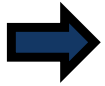
Isolation and Identification of *E. coli*



Tilapia



Pond water



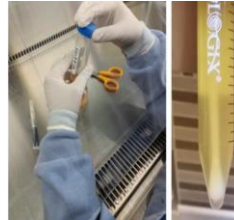
Skin



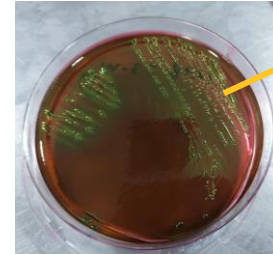
Intestine



Gill

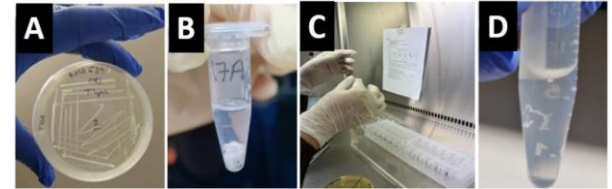


Enrichment: Trypticase soy
broth (TSB)
37° C, 24 h

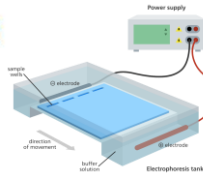


“Metallic green”
color colonies

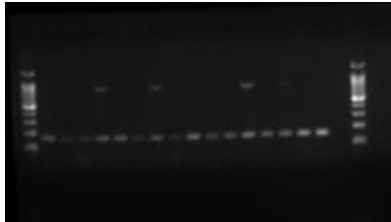
Streaking on Eosin methylene blue (EMB) and
incubation 37° C, 24 h



Genomic DNA (gDNA) extraction



PCR with *E. coli*-specific *uidA*

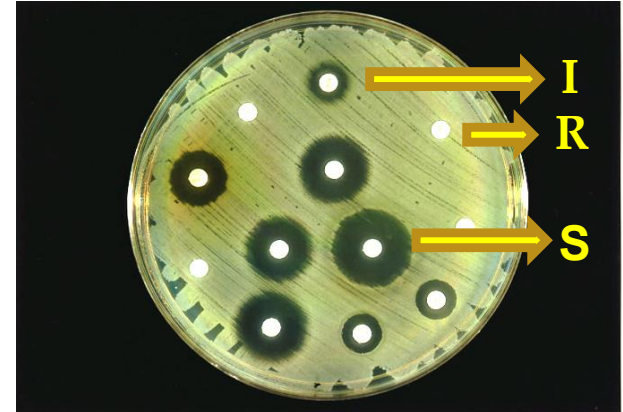


Agarose gel (2%) electrophoresis

Antimicrobial Susceptibility Testing

Disk diffusion test for **14** antimicrobials

- ▶ **Penicillin:** ampicillin (AMP, 10 µg)
- ▶ **Tetracyclines:** oxytetracycline (OT, 10 µg)
- ▶ **Aminoglycosides:** gentamycin (GEN, 10 µg)
- ▶ **Quinolones:** nalidixic acid (NAL, 30 µg), ciprofloxacin (CIP, 5 µg), levofloxacin (LVX, 5 µg), norfloxacin (NOR, 100 µg)
- ▶ **Carbapenems:** meropenem (MEM, 10 µg)
- ▶ **Cephalosporins:** cefuroxime (CXM, 30 µg), cefepime (FEP, 30 µg)
- ▶ **Others:** trimethoprim-sulfamethoxazole (STX, 25 µg), chloramphenicol (CHL, 30 µg), azithromycin (AZM, 15 µg), nitrofurantoin (NIT, 100 µg)



*The results were interpreted as **susceptible (S)**, **intermediate resistant (I)** and **resistant (R)** according to CLSI guidelines*

MAR Index

MAR index signifies the level of AMR in a bacterial isolate and can help **identify high-risk environments** with significant AMU or contamination

$$\mathbf{MAR\ index = a/b}$$

Where

“a” designates the number of antimicrobials the isolate was resistant to

“b” designates the total number of antimicrobials the isolate was tested against

Results

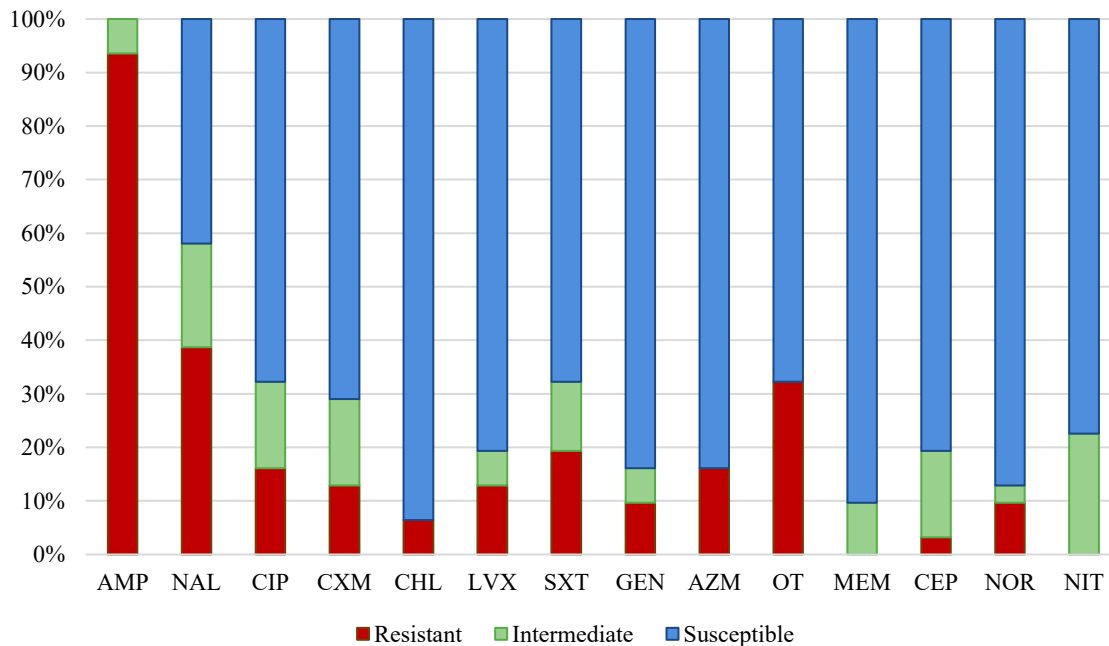
Presence of *E. coli* in tilapia farms

153 biological samples (skin, gills, intestine, and water) from 41 tilapia farms

Source	PCR-positive isolates	Percentage (%)
Intestine	10	32.26
Skin	9	29.03
Pond water	8	25.81
Gill	4	12.90

*Total 31 E. coli (20.26%)
were identified*

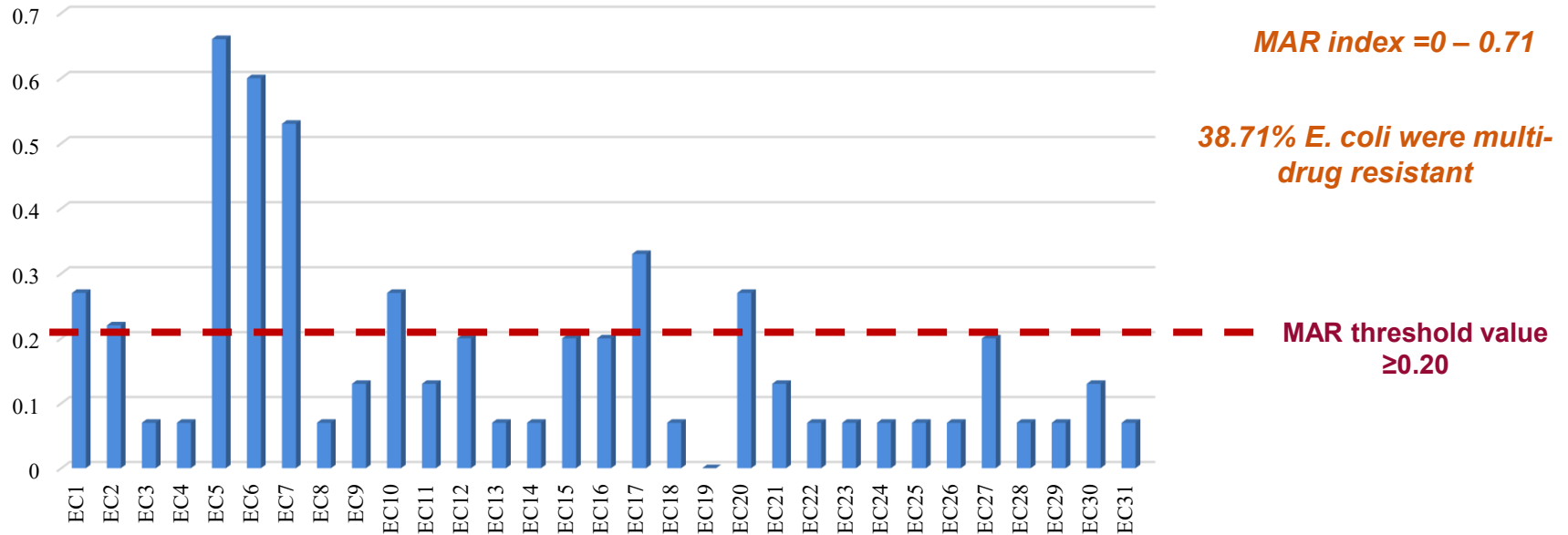
AST Results



High resistance to-

- ✓ **Ampicillin (93.55%)**
- ✓ **Nalidixic acid (38.71%)**
- ✓ **Oxytetracycline (32.26%)**

MAR Index



*A multiple antimicrobial resistance (MAR) index of 0.2 or higher indicates that bacteria originated from a high-risk source of contamination

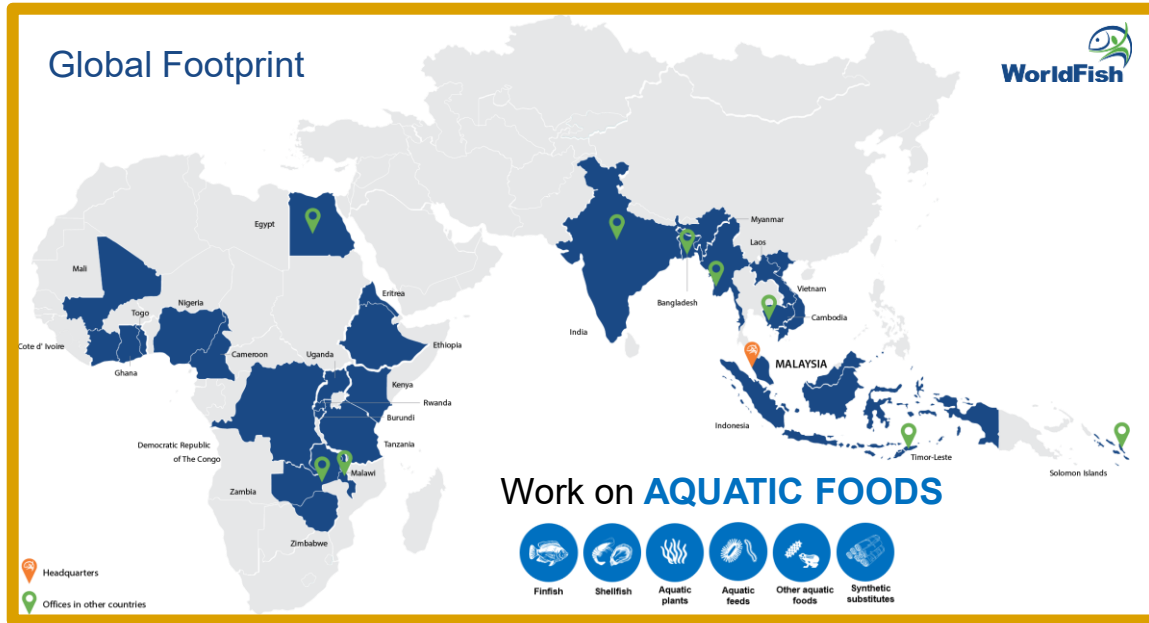
Conclusion

- The study confirms the **low presence of *E. coli*** in fish and environment with low percentage of multidrug resistant strains.
- These findings indicate the **possible risk** AMR transmission through the aquatic food chain.
- **Responsible AMU** is essential to limit the spread of AMR in aquaculture and the environment.
- The way forward is to integrate **molecular innovation**, **ecological insights**, and **stewardship** within a One Health framework.



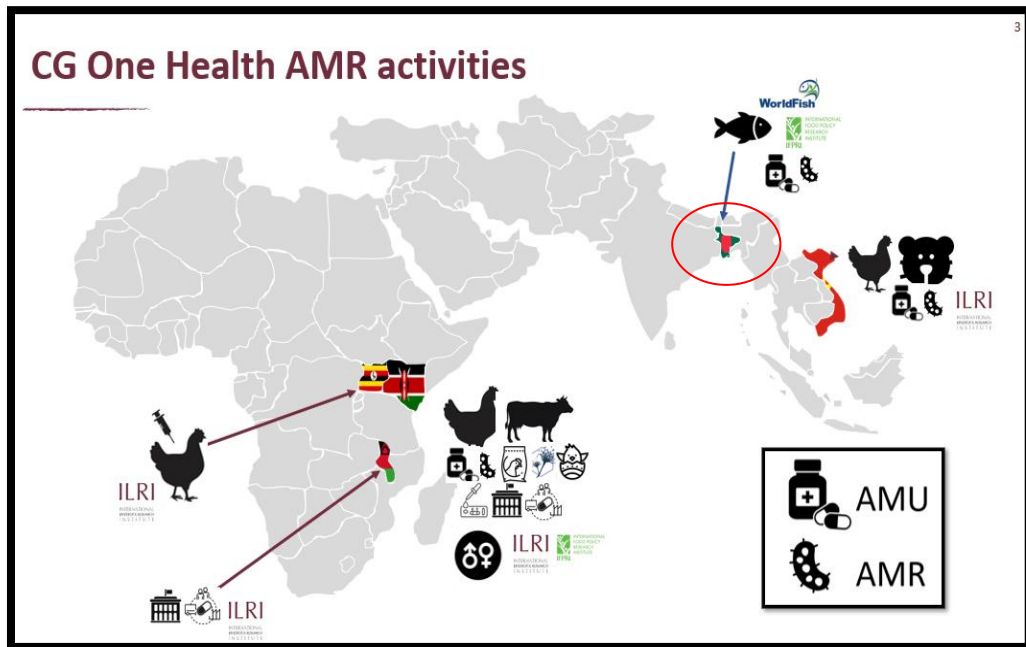
About WorldFish

WorldFish is a non-profit organization under CGIAR, a global research partnership for a food secure future dedicated to reducing poverty, enhancing food and nutrition security, and improving natural resources.



WorldFish is implementing projects in **27 countries** across Asia, Africa, and the Pacific region.

“Sustainable Animal and Aquatic Foods (SAAF)- One Health”



Our team



