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Prevalence and antimicrobial susceptibility characterization of Escherichia coli isolated from the intestines of freshwater fish from the Mindu dam in Morogoro, Tanzania

Ally Mohamed Samiji[1*], Ester Abraham Makyao[1], Frida Richard Mgonja[2], Abdul Seleman Katakweba[3]

Abstract

Background: The Mindu dam, an open system, serves as a microbial reservoir, housing uropathogenic microorganisms like Escherichia coli. Morogoro municipality relies on the dam for water and fish. This study aimed to assess the antimicrobial susceptibility of Escherichia coli isolated from fish and water in Mindu dam.

Methods: In May 2019, a cross-sectional study was conducted in Morogoro municipality. Selected antimicrobials, including ampicillin, tetracycline, cefoxitin, erythromycin, trimethoprim, gentamicin, enrofloxacin, and nalidixic acid, were tested using the disc diffusion method. E. coli ATCC 25922 served as a positive control for quality assurance during bacterial isolation. The potential source of antimicrobial contamination was identified through the multiple antibiotic resistance index.

Results: Out of 148 samples, E. coli was found in 24, none in water. E. coli showed high resistance (≥50.0%) to erythromycin (62.5%), nalidixic acid (79.2%), and ampicillin (75%). Additionally, 58.0% of isolates displayed multi-drug resistance across antimicrobial classes, with a multiple antibiotic resistance index ranging from 0.00 to 0.75.

Conclusion: Antimicrobial-resistant E. coli in the food chain may heighten the spread of complex urinary tract infections in the municipality. It underscores the necessity for robust municipal surveillance of antimicrobial resistance and effective antimicrobial stewardship for enhanced infection prevention and control.

Keywords: Antimicrobial Resistance, Escherichia Coli, MAR Index, Fish, Public Health, Veterinary Health, Mindu Dam, Morogoro Municipality, Tanzania

Correspondence: Ally Mohamed Samiji (alysamiji@gmail.com)
[1]Department of Wildlife Management, Sokoine University of Agriculture, P. O Box 3073, Morogoro, Tanzania

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Background

Fish consumption is increasingly in public to date, as a result of rapid human population growth which increases demand for aquatic products. Fish consumption is largely seen as a positive health behavior since fish offers several kinds of health benefits [1–4], such as proteins, omega-3 fatty acids, and essential vitamins. Nevertheless, fish are reservoirs of zoonotic pathogens that can infect both animal hosts and humans who are in contact with the aquaculture facility via foodborne infections [5,6], unfortunately, these zoonotic pathogens may contain and spread antimicrobial resistant (AMR) genes. Thus, improperly handled fish can expose consumers to infectious bacteria (eg E. coli), including difficult-to-treat multidrug-resistant pathogens (superbugs) [2], yet, increasing threat to human health. It is obvious that antibiotic-resistant strains are now being detected and the spread of these strains could greatly suppress medical treatment options available and increase mortality from previously curable infections [6]. Antimicrobial resistance in bacteria basically occurs as a result of antibiotic modification or destruction, reduced antibiotic accumulation (increase efflux or decrease permeability), and target alterations and may be associated with global adaptation of the bacterial cell [7]. In particular, E. coli resistance is mostly mediated by extended-spectrum ß-lactamase (ESBLs) [8]. Antimicrobial resistance occurs naturally but can be facilitated by the inappropriate use of antimicrobials [7]. The prominent resistance genes that can be identified in fish are those that encode trimethoprim [dfrA1,

dfrA5, dfrA7, dfrA12, and dfrA15], tetracycline [tetA(A) and tetA(G)], erythromycin resistance [mefA], streptomycin [strA-strB], chloramphenicol [cat-1] and amoxicillin [bla (TEM)] to mention a few [9]. These genes can spread to humans through raw or undercooked fish as well as the use of contaminated water. Resistance occurs as a result of natural processes and widespread anthropogenic activities such as the use of a wide variety of antimicrobials in agriculture, veterinary, aquaculture, and human sectors [10,11]. Mindu dam receives water from Uluguru mountains via the Ngerengere river, Mgeta river, Mzinga river and Mlali river, and efflux via Ngerengere river. There are a lot of human activities conducted uphill, along the river and around the dam, notable example includes farming, grazing and fishing, hence, all discharges (including fecal materials) are poured into the dam. Thus, making the aquatic environment a sink pool to various microbes such as E. coli, Staphylococcus aureus, Streptococcus sp. Moreover, aquatic pathogenic bacteria spread more easily by water from fish to fish than terrestrial pathogenic bacteria transfer by air [9,12]. The bacterium E. coli is widely used as an indicator of the biological condition of food and environments due to its almost exclusively fecal origin [13,14]. Like other parts of the country, E coli is the major cause of Urinary tract infections (UTIs) in the Morogoro municipality and the disease incidence tend to increase annually. Similarly, the wide usage of Mindu water and fish across Morogoro municipality might play a role in E. coli transmission. To date, there is no data on the AMR status of E. coli isolates recovered from freshwater fish from the Mindu dam. Therefore, the aim of the current study was to determine antimicrobial susceptibility of E. coli isolates recovered from fish intestines and water (food chain) from the Mindu dam in order to safeguard human health. The findings herein enlighten the potential spread of resistant E. coli to human via the food chain as well as likely to accelerate transmission of complicated UTIs in the municipality. In that sense, the findings call for immediate control strategies in the municipality.

Methods

Study site

The study was conducted in Morogoro municipality, specifically in the Mindu dam. Mindu dam is a man-made dam located at latitudes 6° 51’ S to 6° 52’ S and longitudes 37° 30’ E to 37° 40’ E, with an altitude of 500 m above sea level, southeast of Ngerengere river. The dam is found about 10 km from Morogoro Municipality along the Morogoro-Iringa highway in Eastern Tanzania, and has an area of 303 km2 which lies on the slope of Uluguru Eastern Arc Mountains [33]. Mindu dam has several influxes from Uluguru mountain namely, Ngerengere river, Mlali river, Mzinga river, Mgeta river and efflux from the dam via Ngerengere river. Mainly the dam supplies about 80% of water in the municipality including Sangasanga and Kasanga villages [15]. The economic activities around the dam mainly includes agriculture, fishing and livestock keeping. Moreover, E. coli is the major cause of Urinary Tract Infection (UTI) in the municipality [32].

Study design and participants

A cross sectional study design was used, whereby fish and water samples were collected from different points inside the dam in May 2019. Indeed, fish samples were collected from the dam using fishing nets and stored in sterile plastic bags, placed in cool box with icepacks and transported to Institute of Pest Management for laboratory processing within 4 hours of collection.

Processing

In laboratory, fish samples were stored at 4°C before being processed. Simply, fish samples were placed on the sterile cutting board, identified and wiped with 70% ethanol using cotton wool to decontaminate bacterial load on the skin surface. Each fish sample were dissected individually, sterile forceps was used to remove the intestine under aseptic condition. Similarly, Water samples were collected in 10 mL tube individually to make a total of 100 tubes, with which make a total of 1 L of water collected. All water samples were collected at a depth of 5-7 cm subsurface from different points inside the dam.

Bacteria isolation and confirmation

Under aseptic condition, a homogenous suspension of fecal content was prepared in buffered peptone water (BPW) and incubated for 24 hrs at 37°C, for enrichment. Similarly, water samples in 10 mL tubes were prepared in BPW, enrichment medium, and incubated at 37°C for 24 hrs. Then, one loopful of each prepared sample were streaked on MacConkey agar, followed by incubation for 24 hours at 37°C. Suspected and pure culture were subjected to biochemical tests namely, Indole test, Methyl-red test, Voges-Proskauer, and Citrate test (IMViC) and Gram-negative staining for confirmation of E. coli isolates. Colonies were considered positive E. coli when Indole and Methyl-red positive, Voges-Proskauer and Citrate negative, and Gram-negative with short rods in the gram staining test. A well-recognized bacterial culture (E. coli, ATCC 25922) was used as positive control for screening and confirmation test of the bacteria.

Antimicrobial susceptibility tests

The E. coli isolates were subjected to the antimicrobial susceptibility test using disc diffusion method as described in Kirby-Bauer protocol [34]. Simply, pure culture of each colony was suspended into a test tube contain sterile normal saline solution, its turbidity was then compared to 0.5 McFarland Standard (Oxoid Ltd, Hampshire, UK). Sterile swab was used to inoculate the bacteria suspension by completely streaking onto prepared Muller-Hinton agar (Oxoid Ltd, Hampshire, UK) plates. Using sterile forceps, antimicrobial discs were applied on the agar plates and incubated at 37°C overnight. The inhibition zone diameter produced around the antimicrobial discs were measured using plastic ruler and interpreted per the Clinical and Laboratory Standards Institute [35]. A total of eight antimicrobial agents commonly used in both human and livestock were used to test for resistance notably, Tetracycline (TE, 30µg), Cefoxitin (FOX, 30µg), Erythromycin (E, 15µg), Penicillin (P, 10µg), Sulpharmethoxazole (STX, 25µg), Gentamycin (CN, 10µg), Enrofloxacin (ENR, 5µg), and Nalidixic acid (NA, 30µg).

Identification of multi-drug resistance (MDR) and multiple antibiotic resistance (MAR) index

Multi-drug resistance is well-defined as resistance to more than two antibiotics classes [36]. The MAR index was calculated for each isolate using the formula below, whereby, “a” is the number

of antimicrobials to which a particular isolate was resistance, "b" is the total number of antimicrobials to which isolate was exposed. The MAR index > 0.2 indicate the high-risk source of contamination where antimicrobials are often used [37].
MAR index = a/b

Statistics analysis

Raw data and laboratory results were recorded into Microsoft Excel, cleaned, organized and analyzed. Descriptive statistics were used to analyze percentage, proportion and prevalence.

Results

Sample collected and Occurrence of E. coli isolates

A total of 148 samples were collected, whereby 100 and 48 accounted for water and fish samples respectively. Of 48 fish samples, 35 were Tilapia and 13 were Catfish. Moreover, out of 148 samples only 24 were positive for E. coli bacteria, which entail 19 Tilapia and 5 catfish (Table 1). Most importantly, overall fish contamination frequency was 50% (24/48), specifically, tilapia had high contamination frequency followed by catfish, 54.3% and 38.5% respectively. Interestingly, no E. coli bacteria were observed in all 100 water samples.

Table 1: Occurrence of E. coli bacteria from water and fish intestine collected from Mindu dam, Morogoro municipality.

Sample type	Category	No. samples	E. coli positive samples
Water		100	0 (0.0%)
Fish	Tilapia	35	19 (54.3%)
	Catfish	13	5 (38.5%)
Total		148	24

Antimicrobial susceptibility profile

All E. coli isolates were tested for phenotypic resistance, the results showed variation in resistance status among tested antimicrobial agents and isolates source. Of all tested isolates 95.8% (23/24) were resistance to at least one antimicrobial agent (Table S1). Overall, high resistance rates (≥50%) were recorded for ampicillin (75%), erythromycin (62.5%), and nalidixic acid (79.2%) and extremely low resistance rates (<10%) were observed for tetracycline, gentamicin, enrofloxacin (Figure 1).

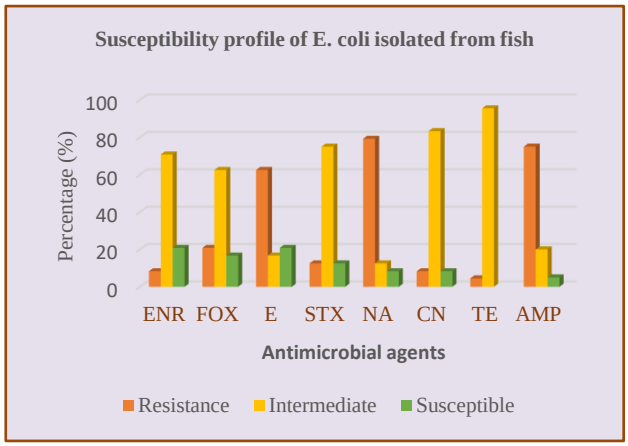

Figure 1: Susceptibility profile of E. coli isolated from the intestines of freshwater fish to selected antimicrobials

Multi-drug resistance (MDR) and MAR index

Multi-drug resistance (MDR) was observed in 58% (14/24) sample isolates, with 11 E. coli isolates from Tilapia and three from Catfish. Higher MDR were observed in three antimicrobial classes namely E-AMP-NA of which 50% (7/14) sample isolates shows MDR to it (Table 2). The MAR index ranged from 0.00 to 0.75, with an average of 0.33 (Table S1). Moreover, 70.8% of tested isolated were found having MAR index > 0.2 and 29.2% having ≤ 0.2 (Figure 2).

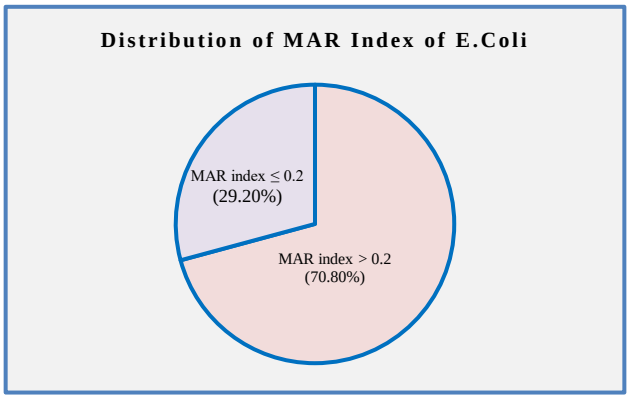

Figure 2: Distribution of MAR index indicating previous exposure of the E. coli to antimicrobial agents (MAR index >0.2), and without likelihood of exposure (MAR index ≤ 0.2).

Table 2: Multidrug resistance (MDR) pattern of E. coli isolated from freshwater fish intestines, Mindu dam, Morogoro municipality.

Source of *E. coli* isolates	Antimicrobial classes resisted	MDR pattern	Number of sample isolates showing MDR
Tilapia	3	E-AMP-NA	7
		FOX-AMP-NA	1
	4	E-STX-AMP-NA	1
		FOX-E-AMP-NA	1
	6	E-STX-AMP-CN-ENR-NA	1
Catfish	3	FOX-E-CN	1
	4	FOX-E-AMP-NA	1
	5	TE-FOX-E-STX-ENR	1

Discussion

Out of 148 total collected samples, 100 were water samples and 48 were fishes. Two types of fish were observed with the majority of Tilapia followed by Catfish, 35 and 13 respectively. This result is in line with Mgode et al. [15], that Tilapia and Catfish are the predominant fish species in Mindu dam. Remarkably, these species are the most commonly marketed in Morogoro municipality. Of 48 total collected fishes, only 24 fishes which constitute 19 Tilapia and 5 Catfish were found with E. coli bacteria in the intestines. Detection of E. coli from fish was not a new phenomenon as it is reported elsewhere [16–18], also the pathogen is common to freshwater fish and known as food borne E. coli that play a role in cases of uropathogenic infections in humans [19]. On the other hand, no E. coli bacteria were isolated from all water samples. The absence of the E. coli isolates in sampled water could be attributed to sampling points, in this study water samples were collected at the middle of the dam.

Table S1: MAR index and Susceptibility profile (Susceptible S, Intermediate I, Resistance R) data from disc diffusion test of E. coli bacteria isolated from fish intestines, Morogoro, Tanzania

		Antimicrobial agents								
Isolate source	Isolate code	ENR	FOX	E	STX	NA	CN	*TE	^AMP	MAR index
Tilapia	T1	S	S	R	S	R	I	S	R	0.37
	T2	S	S	I	S	R	S	S	R	0.25
	T3	S	I	R	S	R	S	S	R	0.37
	T4	S	I	R	S	R	S	S	R	0.37
	T5	S	S	I	S	R	S	S	R	0.25
	T6	S	S	R	S	R	S	S	R	0.37
	T7	S	R	I	S	R	I	S	R	0.37
	T8	S	S	R	R	R	S	S	R	0.5
	T9	I	S	S	S	R	S	S	S	0.12
	T10	S	S	R	S	R	S	S	R	0.37
	T11	S	S	R	S	R	S	S	R	0.37
	T12	R	S	R	R	R	R	S	R	0.75
	T13	S	I	I	S	R	S	S	R	0.25
	T14	S	S	S	S	R	S	S	S	0.12
	T15	S	S	S	S	I	S	S	S	0
	T16	I	S	R	I	R	S	S	R	0.37
	T17	S	S	I	S	R	S	S	I	0.12
	T18	S	R	R	I	R	S	S	R	0.5
	T19	S	S	S	S	R	S	S	S	0.12
Catfish	C1	I	R	R	S	R	S	S	R	0.5
	C2	R	R	R	R	S	S	R	-	0.71
	C3	I	I	R	S	I	S	-	-	0.17
	C4	S	S	R	S	S	S	-	-	0.17
	C5	I	R	R	I	S	R	S	-	0.42

IS: isolate status; S: Susceptible; I: Intermediate; R: Resistant; TE: Tetracycline; FOX: Cefoxitin; E: Erythromycin; STX: Sulpharmethoxazole/Trimethoprim; AMP: Ampicillin; CN: Gentamicin; ENR: Enrofloxacin; NA: Nalidixic acid; *Three catfish isolates tested for TE; ^One catfish isolates tested for AMP

This supports the previous findings that onshore has higher concentration of E. coli and lower concentration at the middle of the dam [20]. Also, [21] reported that E. coli persist longer in sediments than in water columns. Thus, this indicates that Fish from the Mindu dam feed on contaminated benthic fauna and aquatic plants as reported elsewhere [20]. his study revealed a higher E. coli contamination frequency 50% in fish compared to 12.5% of [18] in Tanzania, as well as 23.5% (raw fish) of [22] in northwest Ethiopia. Such an increase in the prevalence of E. coli pathogen in food animals (fish) promotes transmission to humans through the food chain [18,23], hence likely to accelerate uropathogenic infections (such as urinary tract infections UTIs) in the municipality. Interestingly, in Uganda Agoba et al. [24] reported a higher prevalence 83.3%, than what we found. These differences could be due to different sample sources, sizes, methods used, and rate of contaminations of the dams by anthropogenic activities, use of organic manure from farms, and antimicrobial use in the study area. Moreover, proportion of tilapia was shown to have high E. coli contamination frequency 54.3%, compared to catfish 38.4%. This finding is line with previous research that reported a high E. coli prevalence of 22.1% and 19.6% in tilapia and catfish respectively [25]. This could be due to different exposure in-term of habitat, Tilapia prefer shallow water mainly near onshore while Catfish prefer benthic zone. As reported by Korajkic et al. [21] E. coli persist longer in sediments than in water columns. Also, Mhongole et al. [20] reported high concentration of E. coli onshore than in water column. Thus, expose E. coli differently in water. In addition, other studies reported the presence of E. coli in gills, meat, and on the body surface of the fish, as well as in cooked fish [14,22], this speaks to the vulnerability of fish handlers and consumers to E. coli pathogen and its associated diseases. Also, E. coli have been isolated from different sources such as human specimens, foods, aquatic environments, livestock and poultry [16,19,23,26]. Simply, this shows the wide range the pathogen can invades and causes infection. Of all tested isolates 95.8% were resistance to at least one antimicrobial agent. Overall, the E. coli isolates showed higher resistance to Nalidixic acid (79.2%) followed by Ampicillin (75%), and Erythromycin (62.5%). The higher resistance in Ampicillin (75%) has been reported elsewhere [27], Nalidixic acid and Erythromycin have been also reported to be (63.6%) and (72.7%) respectively [16]. Higher resistance to antimicrobials could be due to frequent usage of these antimicrobials as are easily available without prescription from drug outlets for humans and livestock use. Furthermore, 58% of isolates showed MDR, however, the isolates exhibited different resistance pattern. Higher MDR were observed in three combined antimicrobial classes namely E-AMP-NA. This speaks the vulnerability of these drugs on the treatment of bacterial diseases. MDR E. coli have been reported elsewhere [28]. Most importantly, MAR index > 0.2 was observed in 70.8% sample isolates. This informs the previous exposure of the isolates to antimicrobials. Thus, argue against the frequent use of these antimicrobials in human and livestock production. In addition, Mindu dam receives rainwater runoff carrying waste containing antimicrobial residues and fecal materials. So far, the presence of the resistant E. coli bacteria in fish samples is an indication of contamination. This contamination of food with zoonotic enteric pathogens E. coli could accelerate a variety of illnesses to human such as UTIs and gastrointestinal infections. Yet, the increased prevalence of antimicrobial drug resistant organisms in food animals and animal products including livestock, poultry, and fish promotes transmission to

humans through the food chain [18,23]. Further, resistant infections could accelerate morbidity and prolonged hospital stays, as well as infectious periods [19,23,29]. This phenomenon is prevalent in developing countries like Tanzania where a second-line treatment regimen is expensive and less likely for patients to have access [30]. Worth noting that UTI is the foremost causes of morbidity (29.1%) followed by Malaria (23.1%) in the Morogoro region [31]. Furthermore, it has been reported that E. coli is the major cause of UTI in the Morogoro municipality [32]. Thus, make AMR in E. coli of particular concern in safeguarding public health and animal production.

Conclusion

The resistant E. coli isolates from fish food observed in the current study pose a public health risk. Therefore, this study declares the high risk of antimicrobial drug resistant E. coli transmission to humans via the food chain, thus, making the health risk-line longer from fish handler to consumer. Hence, likely to accelerate uropathogenic infections such as UTIs in the Municipality, increase morbidity and make the treatment complicated. Moreover, regardless of the pathway, the transfer of antimicrobial drug resistant pathogens from the environment to fish, humans, and animals have a detrimental effect on human health, and this potential link between the aquatic and terrestrial resistors is of particular concern. Since it facilitates the transfer of resistant pathogens from the environment to the food chain, thus, exposing the public to complicated health threats. Therefore, there is a need to develop active surveillance system to monitor and regulate the use of chemicals and antimicrobials of human and veterinary importance, as part of antimicrobial stewardship. Lastly but not least, further studies are needed on the genetic relatedness among isolates from different sources and the virulence factors of the isolates must be investigated in detail.

Abbreviation

AMR: Antimicrobial Resistant; IL-10: Interleukin 10; IL-8: Interleukin 8; ESBLs: Extended-Spectrum ß-Lactamase; UTIs: Urinary tract infections; MDR: Multi-Drug Resistance; MAR: Multiple Antibiotic Resistance; BPW: Buffered Peptone Water; SUA: Sokoine University of Agriculture; IPM: Institute of Pest Management

Declaration

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Availability of data and materials

Data will be available by emailing alysamiji@gmail.com

Authors' contributions

All authors are equality conceived and designed the study, analyzed and interpreted the data; drafted the manuscript; revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

We conducted the research following the declaration of Helsinki. The ethical approval was obtained from the Ethics Review Committee of Sokoine University of Agriculture [Ref No. SUA/ADM/R.1/8/772 of December 11, 2021] Morogoro, Tanzania.

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interests.

Author Details

[1]Department of Wildlife Management, Sokoine University of Agriculture, P. O Box 3073, Morogoro, Tanzania

[2]Department of Veterinary Physiology, Biochemistry and Pharmacology, Sokoine University of Agriculture, P. O Box 3511, Morogoro, Tanzania

[3]Institute of Pest Management, Sokoine University of agriculture, P. O Box 3110, Morogoro, Tanzania

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