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PROFILING OF ANTIMICROBIAL RESISTANCE (AMR) AND MULTI DRUG RESISTANCE (MDR) OF *ESCHERICHIA COLI* ISOLATES FROM LIVESTOCK, WILD ANIMALS AND AN ORNAMENTAL FISH FARM IN POLGAHAWELA, SRI LANKA

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SUMMARY: The pattern of antimicrobial resistance (AMR) and the prevalence of multi drug resistance (MDR) of *Escherichia coli* (*E. coli*) isolates from livestock, wild animals and an ornamental fish farm were investigated in a selected area in Polgahawela, Sri Lanka. Sampling area of one square Km containing an ornamental fish farm, a broiler farm, cattle, goat, and backyard poultry which are located separately within the sampling area but not in an integrated farming system with its associated wildlife was mapped using GPS. Faecal samples were examined from 89 wild animals (terrestrial and avian species), 28 livestock animals (including poultry) as well as 40 samples from an ornamental fish farm (fish faecal material, pond water and pond sediment). *E. coli* was isolated using standard methods and AMR profiles were investigated for 12 antimicrobials using Kirby-Bauer disc diffusion method. Sixty nine percent (62/89) of wild animals and 86% (24/28) of livestock samples were positive for *E. coli* whereas the isolation rate was 17.5% (7/40) in the samples from the ornamental fish farm. A maximum of two randomly selected *E. coli* isolates from each sample, making a total of 164 isolates were tested for AMR of which 114, 43 and 7 were from wild animals, livestock and the ornamental fish farm, respectively. The *E. coli* isolates tested against 12 antimicrobials reflected that the proportions of *E. coli* isolates resistant to at least one antimicrobial were 30%, 63% and 71% in wild animals, livestock and the ornamental fish farm, respectively. Multidrug-resistant *E. coli* isolates were detected in wild animals, livestock and the samples from ornamental fish farm. Overall, according to the statistical analysis, *E. coli* isolated from livestock and the ornamental fish farm reflected a significantly higher chance of carrying AMR compared with *E. coli* from wild animals.

KEYWORDS: Antimicrobial resistance, Multidrug resistance, *E. coli*, Wild animals, Livestock, Poultry & Ornamental fish

INTRODUCTION

Antimicrobial resistance (AMR) has now been declared a global emergency by the World Health Organization (WHO) due to the associated mortalities and high treatment costs linked to diseases caused by resistant microorganisms. Overuse and misuse of antimicrobials in human and veterinary medicine have accelerated the emergence of AMR while the challenges in developing new antimicrobials have engraved the AMR issue (Costanzo and Roviello, 2023; Rehman, 2023). The surveillance of AMR is a crucial step in addressing this issue methodically. Many surveillance programmes are conducted worldwide to study and evaluate the extent of AMR distribution (Ma *et al*., 2023; Sherry *et al*., 2023; Wilson *et al*., 2023). According to the limited published data available, Sri Lanka too has been challenged with the AMR issue (Gunasekara *et al*., 2023). With the aim of

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combating the AMR problem, a National Strategic Plan (2017-2022) with multi sectoral collaboration has been launched. Guidelines for the rational use of antibiotics with national guidelines on therapeutic and prophylactic use of antimicrobials have been developed accordingly (*National Strategic Plan for Combating Antimicrobial Resistance in Sri Lanka*, 2022).

An ecosystem is a biological community with its physical and chemical environment and the dynamic interactions of nutrient cycles and energy flows that link those (Loreau, 2010). There are various ecosystems based on the differences in the biological community and physical and chemical environment. However, the most basic categorisation involves three habitats; terrestrial, marine, and aquatic. Terrestrial ecosystems are land based, while aquatic and marine are water-based. Animals are a key component of an ecosystem (Wang and Zhai, 2019). This includes farming animals and wild animals. It is known that antimicrobials are routinely used in intensive livestock farming (Hosain, 2021; Tiseo *et al*., 2020). In intensive poultry farming, the animals are reared with the aim of producing maximum yields with low cost of production and higher profit. Entire groups of animals are mass medicated and antibiotics are given prophylactically for disease prevention. Therefore, this opens the platform for a high demand for antimicrobials and lead to the spread of antimicrobial resistance with environmental pollution. Admittedly, antimicrobials are not used in wild animals but they may get exposed to resistant bacteria and resistant genes from contaminated ecosystem due to their extensive home range and distinctive feeding and defaecation patterns. As a result wild animals can acquire antimicrobial resistance and can act as reservoirs with a biological mechanism to disseminate antimicrobial resistance into a wide range of environmental niches (Radhouani *et al*., 2012; Ramey, 2021).

Furthermore, the Antimicrobial Use (AMU) has an impact on the distribution of AMR phenotypes and resistance genes (Hao *et al*., 2014; Hosain, 2021). As a result of the variety of AMU practices for different species in different ecosystems, AMR phenotypes and genotypes in one ecosystem may not be representative of those in another (Gow *et al*., 2008). Therefore, in understanding the epidemiology of AMR, describing AMR throughout all phases of all ecosystems is a vital aspect.

Escherichia coli (*E. coli*) and commensal enterococci are regarded as important indicators for assessing the prevalence of resistance in various populations for the purpose of identifying the movement of resistant bacteria between populations both within and between ecosystems (Anjum *et al*., 2021). *E. coli* can thrive in a wide range of conditions and has been shown to colonise a wide variety of mammals including birds. Due to faecal contamination or contamination during the slaughter of food animals, it is frequently detected in the intestinal systems of humans and animals as well as in soil, water, and foods (Momtaz *et al*., 2012). This offers a pool of genes for antimicrobial resistance that can contribute to the epidemiology of the spread of resistance (Alonso *et al*., 2016; Sheikh *et al*., 2012). *E. coli* isolated from fish and the associated aquatic environment is useful in determining the level of *E. coli* contamination in the intestinal contents of fish species and the associated aquatic environments (Cloete *et al*., 1984; Thi and Dang, 2012).

The practice of livestock production is linked to a multitude of landscape changes, many of which can affect wildlife. These consequences are complicated by nature and are influenced by the environment and the form of animal production. With the increasing demand, land that is currently occupied by forests and other natural vegetation is converted to support livestock production (Foley *et al*., 2005). Wildlife populations are under threat due to this change in land use and tend to share the same land with livestock resulting in many issues regarding space, food, disease control including the spread of AMR (Gordon, 2018; Schieltz and Rubenstein, 2016). Hence, in the current study, an area with distinguished interaction patterns with livestock and wild animals has been selected to study the occurrence of AMR resistant *E. coli*. The study area is composed of an ornamental fish farm, a broiler farm, cattle, goat and backyard poultry which are located separately within the sampling area coexisting with its associated wildlife. In the current study, poultry is categorised under livestock.

MATERIALS AND METHODS

Sample Collection

One square Km area at Waddeniya, Polgahawela containing an ornamental fish farm, a broiler farm, cattle, goat, and backyard poultry with its associated wildlife was mapped using GPS as the sampling area (Figure 1).

Collection of faecal samples from wild animals and livestock

Voided faecal samples from wild animals and livestock (poultry, cattle and goat) were collected into a sterile polythene bag and were transported to the laboratory in a cool box immediately after collection (Table 1). Identification of wild animal species and collection of respective voided faecal samples from wild animals, were done by a trained team under the supervision of the accompanied wildlife veterinary surgeon.

Collection of samples from the ornamental fish farm

Fish faecal material, pond water and pond sediment samples were collected from the ornamental fish farm. For fish faecal samples, three different fish from four different species (Angel, Gold Fish, Platy and Guppy) were collected from three different tanks. Live fish were transported to the laboratory and each species was kept in one tank. In the laboratory, freshly voided feces (from individual fish) were cultured on MacConkey agar.

Tanks for the collection of pond sediments were selected to include those with high stocking densities, high suspended solids and algae and with polyculture (different species in a single tank). Pond water samples were collected from the same tanks from which the sediment samples have been collected. Pond sediment and water samples were collected into 50 ml sterile centrifuge tubes and were transported to the lab in a cool box, centrifuged and cultured on MacConkey agar (Table 1).

Table 1. Sample collection from the sampling area of Waddeniya, Polgahawela.

Category	Number of samples collected
Wild animals	
Terrestrial	
Wild boar (*Sus scrofa*)	01
Common palm civet cat (*Paradoxurus hermaphrodites*)	08
Toque monkey (*Macaca sinica*)	04
Porcupine (*Hystrix indica*)	01
Hare (*Lepus nigricollis*)	05
Fishing cat (*Prionailurus viverrinus*)	01
Mongoose (*Urva smithii*)	03
Frog (*Pseudophilautus asankai*)	03
Giant African snail (*Achatina fulica*)	06
Brown fish owl (*Ketupa zeylonensis*)	01
Avian	
Yellow billed babbler (*Argya affinis*)	16
Common king fisher (*Alcedo atthis*)	02
Magpie robin (*Copsychus saularis*)	01
Sri Lanka Myna (*Gracula ptilogenys*)	04
Brown headed barbet (*Megalaima zeylanica*)	01
Water hen (*Gallinula chloropus*)	01
Brown wood owl (*Strix leptogrammica*)	03
House crow (*Corvus splendens*)	03
Spotted dove (*Spilopelia chinensis*)	01
Greater coucal (*Centropus sinensis*)	01
Sri Lanka drongo (*Dicrurus lophorinus*)	01
Plain prinia (*Prinia inornata insularis*)	02
Great egret (*Ardea alba*)	06
Birds (unidentified species)	14
Livestock	
Poultry	15
Cattle	10
Goat	03
Ornamental Fish Farm	
Fish faecal	12
Pond water	14
Pond sediment	14
Total	157

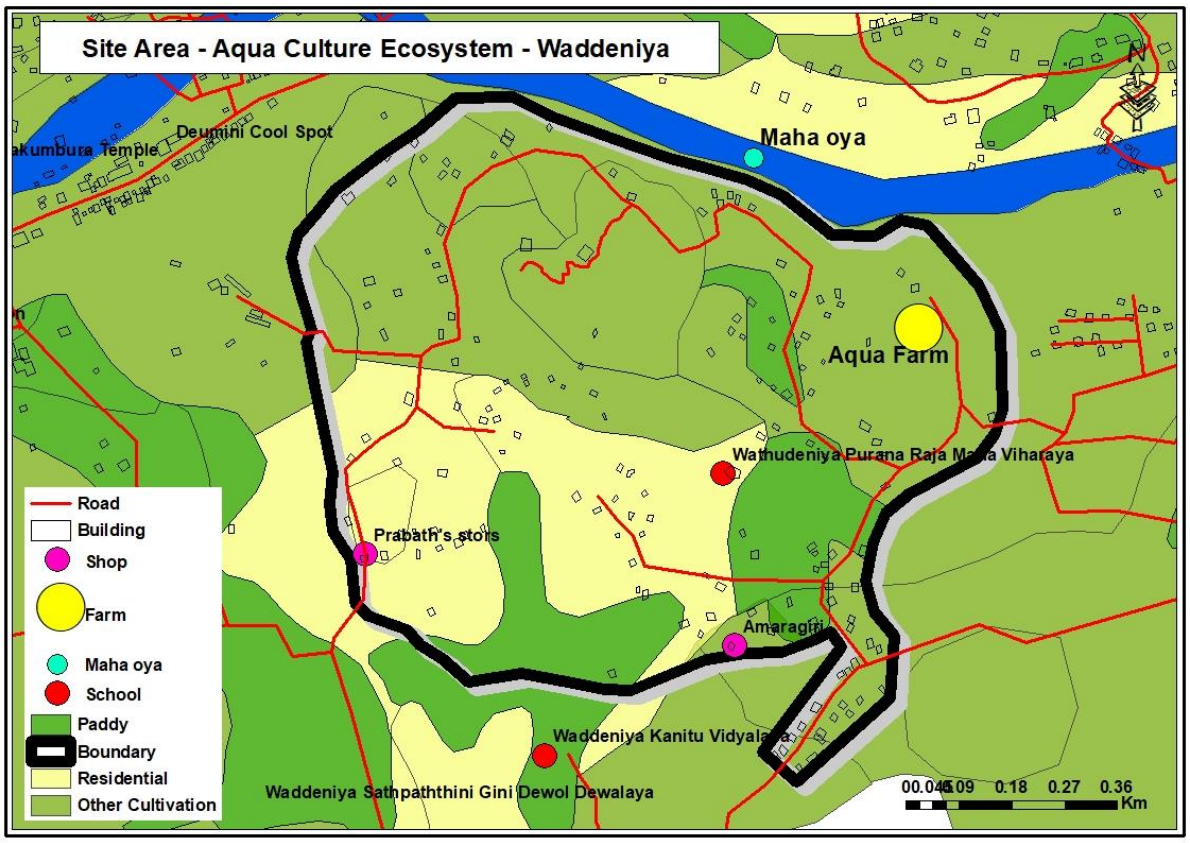

Figure 1. One square kilometer area at study site mapped using GPS. The Boundary indicates the designated one square km a sample collection area. Maha oya – Water canal

Laboratory Methods

All the collected samples were transported to and analysed at the Food Microbiology Laboratory of the Department of Veterinary Public Health and Pharmacology, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya. Overall, from the faecal and other samples, *E. coli* was isolated using established standard laboratory procedures (Markey *et al*., 2013; Bamunusinghage *et al*., 2019) . Thereafter, *E. coli* isolates were tested to examine their phenotypic AMR profiles using the Kirby- Baur disk diffusion method following CLSI guidelines (2020 VET01S-Ed5). Based on the generated phenotypic data, the prevalence of AMR and MDR in *E. coli* isolates from wild animals, livestock and the ornamental fish farm were evaluated. The study was conducted from September 2020 to July 2021.

Isolation and identification of E. coli

Analysis of faecal samples and pond sediment

Each whole faecal samples from livestock, wild animals and fish were mixed well using a sterile cotton swab and directly inoculated on a MacConkey agar plate. Pond sediment and pond water samples were centrifuged and supernatants were removed. The sediment was mixed, a suspension was made and loopful taken from the suspension was cultured on MacConkey agar plates. After incubation of MacConkey agar plates at 37 º C for 24 hours, two presumptive *E. coli* colonies (lactose positive; bright pink) on MacConkey agar were selected for gram staining and oxidase testing. Gram negative rods which are oxidase negative were streaked on nutrient agar (NA) to obtain a pure culture. Cultures on NA were cultured on Eosin Methylene Blue (EMB) agar and the colonies having a unique and characteristic metallic sheen were subjected to Citrate, TSI, Urease and Indole tests for the identification of *E. coli* (Markey *et al*., 2013). All the identified *E. coli* isolates were stored at -80 º C for further investigations.

Phenotypic detection of AMR profiles of E. coli

Maximum of two *E. coli* isolates isolated and identified from each sample were tested for their AMR profiles using Kirby- Baur Disk Diffusion Test for 12 clinically important antimicrobials: ampicillin (AMP, 10 µg), tetracycline (TET, 30 µg), gentamicin (CN, 10 µg), ciprofloxacin (CIP, 5 µg),

imipenem (IPM, 10 µg) streptomycin (S, 10 µg), trimethoprim sulphamethoxazole (SXT, 25 µg), nalidixic acid (NA, 30 µg), amikacin (AK, 30 µg), chloramphenicol (C, 30 µg), ceftazidime (CAZ, 30 µg) and cefotaxime (CTX, 30 µg) representing different antimicrobial classes (Table 3) following the guidelines given by Clinical Laboratory Standards Institute (CLSI) 2020 VET01S-Ed5. In brief, Mueller Hinton Agar (MHA) plates were evenly inoculated with a suspension of pure *E. coli* culture onto the surfaces of MHA plates and thereafter different antibiotic disks were placed. After incubation, diameters of the inhibition zones were measured and results were interpreted as susceptible, intermediate and resistant, based on the breakpoints mentioned in CLSI (2020 VET01S-Ed5). Quality Control was performed as per the recommendation of CLSI using the control strain ATCC *E. coli* 25922.

Detection of the prevalence of MDR in E. coli from wild animals, livestock and the ornamental fish farm

Based on the antimicrobial resistance profiles of the *E. coli* obtained above, MDR *E. coli* were identified adhering to the definition of the ability of bacteria to resist at least one antimicrobial in three or more antimicrobial classes (Basak *et al*., 2016).

RESULTS

Prevalence of *E. coli* in samples from wild animals, livestock and the ornamental fish farm

The overall prevalence of *E. coli* in wild animals, livestock and the ornamental fish farm was 69% (62/89), 86% (24/28) and 17.5% (07/40), respectively.

Table 2. Prevalence of *E. coli* from faecal samples of wild animals, livestock and from the ornamental fish farm in the sampling area: Waddeniya, Polgahawela.

Category	No. of samples (N)	No. of samples positive for *E. coli (n)*	Prevalence % (n/N)
Wild animals	89	62	69%
Terrestrial	33	26	79%
Avian	56	36	64%
Livestock (overall)	28	24	86%
Poultry	15	13	87%
Cattle	10	08	80%
Goat	03	03	100%
Ornamental Fish Farm (overall)	40	07	17.5%
Fish (faecal)	12	01	8%
Pond water	14	03	21%
Pond sediment	14	03	21%

Antimicrobial resistance profiles of *E. coli* isolated from wild animals, livestock and the ornamental fish farm.

Maximum of two *E. coli* isolates from each sample making a total of 164 isolates were tested for AMR of which 114, 43 and 7 were from wild animals, livestock and the ornamental fish farm, respectively.

E. coli from wild animals revealed resistance against ampicillin (20.1%), streptomycin (14%), tetracycline (8.7%), trimethoprim/sulfamethoxazole (7%) and nalidixic acid (6.1%). Resistance below 5% was shown by the antimicrobials, chloramphenicol (4.3 %), ciprofloxacin (3.5%), cefotaxime (1.7%) and ceftazidime (1.7%). All the wild animal *E. coli* were susceptible to gentamicin, imipenem and amikacin (Table 3, Figure 2).

E. coli from livestock showed resistance against eleven out of twelve antimicrobials. The highest resistance was for ampicillin (52.1%) followed by tetracycline (39.1%). Streptomycin, nalidixic acid and trimethoprim/sulfamethoxazole reflected 36.9% of resistance and there was 30% resistance to ciprofloxacin. Low levels of resistance (4-18%) were noted for ceftazidime, cefotaxime, chloramphenicol, imipenem and amikacin. Among

07 isolates of *E. coli* from ornamental fish farm, 71.4% resistance was for ampicillin, 57.1%, 42.8% and 14.2% respectively for tetracycline, nalidixic acid and each imipenem, streptomycin and chloramphenicol (Table 3, Figure 2).

Among the forty three *E. coli* isolates from livestock, twenty seven *E. coli* isolates were from poultry. Of the poultry *E. coli* isolates, the highest percentage (70%) was detected against ampicillin followed by nalidixic acid (63%), trimethoprim/ sulfamethoxazole (59%), tetracycline (59%) and streptomycin (55.5%). AMR profiles of poultry *E. coli* against 12 antimicrobials is depicted in Figure 3.

Statistical Analysis

Resistance to at least one antimicrobial agent is used as the prevalence of AMR and the criteria for the statistical analysis as previous literature (Bamunusinghage *et al*., 2022). Thirty percent (34/ 114), 63% and 71% (5/7) of *E. coli* from wild animals, livestock and the ornamental fish farm were resistant to at least one antimicrobial agent tested.

The prevalence of AMR in *E. coli* from wild animals, livestock and the ornamental fish farm were analysed using the chi square test and the Fishers exact test by calculating the P value using the statistical software Minitab. According to the statistical analysis, *E. coli* isolated from livestock and the ornamental fish farm reflected a significantly higher chance of carrying AMR compared with *E. coli* from wild animals (Livestock Vs Wild animals; P= 0.000,Ornamental Fish Farm ^{Vs} Wild animals; Fishers exact test; P= 0.0347). There was no statistically significant difference in the prevalence of AMR in *E. coli* from livestock and the ornamental fish farm (Ornamental Fish Farm ^{Vs} Livestock; Fishers exact test; P> 0.05).

Table 3. AMR profiles of *E. coli* isolated from wild animals, livestock and the ornamental fish farm. The total number of *E. coli* isolates tested (N) = 164

Antimicrobial Agent and Disc Concentration	Antimicrobial Class	Percentage % (n) of Resistant (R) Isolates			Percentage % (n) of Intermediate (I) Isolates		
		Wild animal	Livestock	Ornamental Fish Farm	Wild animal	Livestock	Ornamental Fish Farm
Ampicillin (AMP, 10 µg)	Penicillin	20.1 (23)	52.1 (22)	71.4 (5)	27 (31)	16 (7)	0 (0)
Tetracycline (TET, 30 µg)	Tetracycline	8.7 (10)	39.1 (17)	57.1 (4)	0 (0)	2.3 (1)	14.2 (1)
Streptomycin (S, 10 µg)	Aminoglycoside	14 (16)	36.9 (16)	14.2(1)	57 (65)	37.2 (16)	71.4 (5)
Nalidixic Acid (NA, 30 µg)	Quinolone	6.1 (7)	36.9 (16)	42.8 (3)	3.5 (4)	11.6 (5)	0 (0)
Trimeth/ Sulfa (SXT, 25 µg)	Sulfonamide	7 (8)	36.9 (16)	0 (0)	0.8 (1)	4.6 (2)	0 (0)
Ciprofloxacin (CIP, 5 µg)	Fluoroquinolone	3.5 (4)	30 (13)	0 (0)	6 (7)	11.6 (5)	14.2 (1)
Ceftazidime (CAZ, 30 µg)	Cephalosporin	1.7 (2)	18.6 (8)	0 (0)	0 (0)	0 (0)	0 (0)
Cefotaxime (CTX, 30 µg)	Cephalosporin	1.7 (2)	16.2 (7)	0 (0)	6 (7)	18.6 (8)	0 (0)
Chloramphenicol (C, 30 µg)	Chloramphenicol	4.3 (5)	7 (3)	14.2 (1)	0.8 (1)	7 (3)	0 (0)
Imipenem (IPM, 10 µg)	Carbapenem	0 (0)	2.3 (1)	14.2(1)	5 (6)	9.3 (4)	0 (0)
Amikacin (AK, 30 µg)	Aminoglycoside	0 (0)	4.6 (2)	0 (0)	7 (8)	11.6 (5)	14.2 (1)
Gentamicin (CN, 10 µg)	Aminoglycoside	0 (0)	0 (0)	0 (0)	3.5 (4)	7 (3)	0 (0)

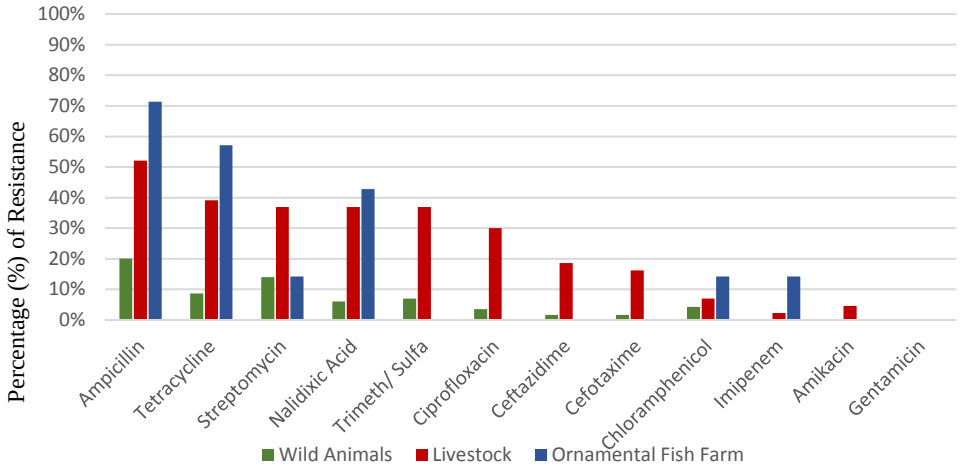

Figure 2. AMR profiles of *E. coli* isolated from wild animals, livestock and the ornamental fish farm. The total number of *E. coli* tested (N) = 164. Trimeth/ Sulfa = Trimethoprim/ Sulfamethoxazole

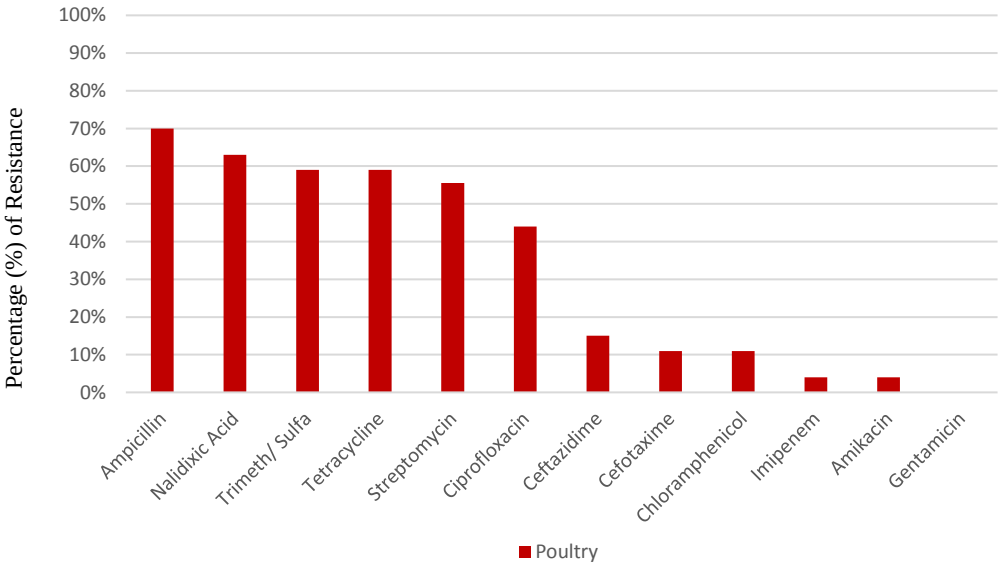

Figure 3. AMR profiles of *E. coli* isolated from poultry faecal samples. The total number of poultry *E. coli* isolates tested = 27. Trimeth/ Sulfa= Trimethoprim/ Sulfamethoxazole

Prevalence of MDR in *E. coli* isolated from wild animals, livestock and the ornamental fish farm.

According to the definition for MDR is the ability of bacteria to resist at least one antimicrobial in three or more antimicrobial classes (Basak *et al*., 2016), of the total of 164 *E. coli* isolates tested, 21% (30/ 164) were MDR. These comprised of 51% (22/ 43), 43% (3/ 7), and 9% (10 / 114) from livestock, ornamental fish farm, and wild animals, respectively. Among the livestock *E. coli* isolates, poultry reflected 70% (19/27) of MDR. All MDRs were resistant to ampicillin while 74% and 52% resistance were observed against tetracycline and streptomycin, respectively. Around 34% of MDRs were resistant for more than five antimicrobials. Ten wild animal MDRs were from a porcupine, a fishing cat and birds (Table 4 and 5).

Table 4. Prevalence of AMR and MDR of *E. coli* isolated from wild animals, livestock and the ornamental fish farm.

Category	Percentage of *E. coli* resistant to at least one antimicrobial agent	Percentage of MDR *E. coli*
Wild animals	34/114 = 30%	10/114 = 9%
Livestock	27/43 = 63%	22/43 = 51%
Ornamental Fish farm	5/7 = 71%	3/7 = 43%
Total	66/164= 40%	35/164 = 21%

Table 5. Resistance phenotypes of MDR *E. coli* isolates

No	Sample	Resistance Phenotypes	No of resistant antimicrobials/ No of antimicrobials tested
1	Poultry 1	AMP TET S CIP IPM SXT NA CTX	8/12
2	Poultry 2	AMP TET S SXT NA CAZ	6/12
3	Poultry 3	AMP TET S CIP SXT NA	6/12
4	Poultry 4	AMP S NA AK	4/12
5	Poultry 5	AMP TET S CIP SXT NA CAZ C CTX	9/12
6	Poultry 6	AMP CIP SXT NA	4/12
7	Poultry 7	AMP TET S CIP SXT NA	6/12
8	Poultry 8	AMP TET SXT NA	4/12
9	Poultry 9	AMP S CIP NA	4/12
10	Poultry 10	AMP S CIP SXT NA	5/12
11	Poultry 11	AMP TET CIP SXT NA CAZ	6/12
12	Poultry 12	AMP TET CIP S SXT NA CAZ	7/12
13	Poultry 13	AMP TET CIP S SXT NA	6/12
14	Poultry 14	AMP TET CIP S CAZ NA CTX	7/12
15	Poultry 15	AMP TET SXT	3/12
16	Poultry 16	AMP TET CIP S SXT NA C	6/12
17	Poultry 17	AMP TET SXT NA	4/12
18	Poultry 18	AMP TET S NA	4/12
19	Poultry 19	AMP CIP SXT NA	4/12
20	Poultry 20	AMP TET S SXT C	5/12
21	Cattle 1	AMP TET CAZ	3/12
22	Cattle 2	AMP S SXT	3/12
23	Fishing Cat	AMP TET CIP S SXT NA C	7/12
24	Bird	AMP TET CIP NA C	5/12
25	Bird	AMP TET SXT NA	4/12
26	Bird	AMP TET SXT	3/12
27	Bird	AMP C CTX	3/12
28	Bird	AMP TET S SXT NA	5/12
29	Bird	AMP TET CIP S SXT NA C	7/12
30	Bird	AMP TET CIP S SXT NA C	7/12
31	Porcupine	AMP S CAZ CTX	4/12
32	Porcupine	AMP S NA CAZ	4/12
33	Pond Water	AMP TET NA C	4/12
34	Pond Sediment	AMP TET NA	3/12

35	Fish Faecal – *Sp*. Angel	AMP	TET	IPM	S	NA	5/12

AMP – Ampicillin, S – Streptomycin, CTX – Cefotaxime, TET – Tetracycline, IPM – Imipenem, CIP – Ciprofloxacin, AK – Amikacin, NA – Nalidixic Acid, CAZ – Ceftazidime, CN – Gentamicin, SXT – Trimethoprim/ Sulfamethoxazole.

DISCUSSION

The current study aimed at the isolation of *E. coli* from livestock, wild animals and an ornamental fish farm within an area of 1 sq. km to profile the AMR and MDR against twelve antimicrobials reflecting nine antimicrobial classes. A higher prevalence of *E. coli* in livestock is detected in the current study (86%) in comparison with the findings of a previous study in Sri Lanka conducted by Bamunusinghage *et al*., (2022) but the prevalence reported herein is comparable to many studies done in other countries (Length, 2012). The prevalence of *E. coli* in wild animals was 69% which is comparatively higher (36%) and lower (76%, 70%) than the previous studies conducted in Sri Lanka (Bamunusinghage *et al*., 2022; Bamunusinghage *et al*., 2019). The geographical location, species and dietary habits have an influence in the isolation rate of *E. coli* from faecal samples (Murphy *et al*., 2021; Ong *et al*., 2020; Priyantha *et al*., 2021).

Even though the origin of the *E. coli* isolated from the ornamental fish farm is not clear, it is indicative of *E. coli* contamination of the ornamental fish farm (Banerjee & Ray, 2017; Dang & Dalsgaard , 2012). It could either be due to human contact with the fish farm in activities such as cleaning, feeding etc, wild animals feeding on fish can expose the fish farm tanks or constitute to the endogenous flora of the fish (Weir *et al*., 2012; Haenen *et al*., 2020).

The composition of fish gut micro biome is complex and it is associated with the fish nutrition and defense mechanisms against diseases. It is not constant and changes according to the nutritional status, age and surrounding environmental factors (salinity of water, bacterial load in the water) (Banerjee & Ray, 2017; Cahill, 1990).

Several research studies have highlighted the presence of different types of bacteria (*aeromonas sp., bacillus sp., enterococcus sp.)* in the marine and fresh water fish gut micro biome of which *E. coli* is not predominant. However, our findings revealed the high percentages of AMR and MDR detected in the *E. coli* from the ornamental fish farm samples. Even though the published data of the AMR *E. coli* in ornamental fish culture is not available in Sri Lanka, *E. coli* isolated from shrimp muscle has revealed considerably high percentages of resistance against the antibiotics of common use. Prevalence of AMR and MDR in *E. coli* isolated from ornamental fish farm samples in the current study and many research done around the world highlight its potential risk in transmission of AMR bacteria to wider environment via the routes of export (Haenen *et al*., 2020; Rose *et al*., 2013). Further, the surface runoff from livestock containing antimicrobial residues and resistant bacteria into fish ponds may encourage the growth and selection of antibiotic-resistant bacteria, with a possible danger of resistance genes spreading into a variety of aquatic environmental microorganisms (Dang *et al*., 2011).

Twenty percent, 14% and 9% of *E. coli* from wild animals were resistant to ampicillin, streptomycin and tetracycline, respectively while 9% was MDR. A few studies conducted in Sri Lanka have shown the prevalence of AMR in wildlife groups. (Bamunusinghage *et al*., 2022; Bamunusinghage *et al*., 2019). Wild animals in the selected area of the current study are not exposed to antimicrobials directly as wild animals are not treated with antimicrobials unlike livestock species. The vast home range, feeding patterns, livestock and human contact may result in exposure to AMR bacteria as well as antibiotic residues therefore, the relatively high prevalence of AMR in wild animal *E. coli* is a significant indicator of environmental contamination of the selected area with AMR bacteria and the possible transmission from livestock to wild animals (Radhouani *et al*., 2012; Ramey, 2021). Wild animals can have direct interaction with livestock and are more likely to come into touch with bacterial resistance genes, commensals and selective agents. Further, it is of significance to highlight the AMR transmission that occurs through environmental dissemination due to residual contamination rather than direct contact with livestock (Cunha *et al*., 2020; Palmeira *et al*., 2021).

Overuse and misuse of antimicrobials in livestock, poultry and aquaculture are still ongoing in Sri Lanka despite the regulations imposed by the government through the Department of Animal

Production and Health (DAPH) (Ariyawansa *et al*., 2023; Dhanapala *et al*., 2021; Priyantha *et al*., 2021). This could serve as a cause for the significantly higher chance of carrying AMR in *E. coli* from livestock and the ornamental fish farm compared with *E. coli* from wild animals according to the statistical analysis. Infections caused by pathogenic bacteria that are resistant to nearly all alternative antibiotics have spread extensively throughout the world, making carbapenems and Extended Spectrum Cephalosporins more and more important. Because of this, multi-drug resistant (MDR) infections in humans are only treated with carbapenems as the last option and the World Health Organization (WHO) has listed the Extended Spectrum Cephalosporins as the antimicrobials of critical importance for human health (Sato *et al*., 2014). Therefore, preventing the spread of carbapenem and cephalosporin resistance in bacteria is of utmost importance. According to the current study findings, resistant and intermediate isolates against imipenem were detected in *E. coli* from livestock, wild animals and ornamental fish farm while resistance against ceftazidime and cefotaxime in livestock and wild animals pose a public health threat and is extreme cause of concern (Suzuki *et al.*, 2009 ; Webb *et al*., 2016 ; Carattoli, 2008). Due to the high demand and profit, poultry production has secured a significant place in the livestock sector in Sri Lanka in comparison with the dairy. High prevalence of AMR and MDR in the poultry production around the globe has become a global concern and surveillance is the key in battling the problem. Researching the patterns of antibiotic usage is difficult in many countries, and the poultry industry in Sri Lanka is no exception (Kiambi *et al*., 2021; Quaik *et al*., 2020). But according to the limited research conducted, amoxicillin, tetracycline and trimethoprim/ sulfamethoxazole are among the most extensively used antimicrobials in poultry sector in Kurunegala district to which the study area, Polgahawela belongs (Ariyawansa *et al*., 2023). Not surprising, in the AMR profiles of *E. coli* isolated from poultry samples, ampicillin, trimethoprim/ sulfamethoxazole and tetracycline are among the top most antimicrobials the organism demonstrated resistance. High prevalence of AMR and 70% of MDR in poultry isolates in the current study is alarming and deeply concerning. Coincidentally, the limited published data conducted on AMR in poultry in Sri Lanka by Priyantha *et al.,* (2021) is similar to our findings in this study.

CONCLUSION

The prevalence of AMR and MDR in *E. coli* isolated from livestock, particularly poultry and the ornamental fish farm is alarming. Further, the prevalence of AMR and MDR in *E. coli* from wild animals is indicative of environmental contamination with AMR and possible transmission of AMR from livestock to wild animals. The prudent use of antimicrobials in human and animal compartments are of equal significance and a collaborative approach for AMR and AMU within the frame of one health should be promoted.

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