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Antimicrobial Resistance Patterns of Fecal *Escherichia coli* in Wildlife, Urban Wildlife, and Livestock in the Eastern Region of Sri Lanka, and Differences Between Carnivores, Omnivores, and Herbivores

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ABSTRACT: Antimicrobial resistance (AMR) is a major public health concern. We identified AMR in fecal *Escherichia coli* of wildlife (WL), urban wildlife (UWL), and livestock in the eastern region in Sri Lanka and compared the prevalence of AMR bacteria from carnivores, omnivores, and herbivores. Fecal samples were collected from 165 animals: WL (*n*=47), UWL (*n*=54), and livestock (*n*=64). *Esherichia coli* was cultured from 129 samples, with isolation rates of 76% from WL (36/47), 70% from UWL (45/54), and 75% from livestock (48/64). Testing *E. coli* isolates against 12 antimicrobials using the disk diffusion method revealed that the proportions of *E. coli* isolates resistant to at least one antimicrobial were WL 52.7%, UWL 20%, and livestock 52%. Multidrug-resistant *E. coli* isolates were detected in WL, UWL, and livestock. Overall, the prevalence of *E. coli* isolates with AMR was significantly lower in UWL compared with WL and livestock. The number of isolates showed AMR was significantly higher in *E. coli* from carnivores than in isolates from omnivores and in herbivores. We conclude that AMR *E. coli* in Sri Lanka is widespread in WL, UWL, and livestock. The higher incidence of AMR bacteria in carnivores compared with herbivores and omnivores suggest that the mechanisms of spread of AMR may vary among wild animals, which requires further investigation.

Key words: Antimicrobial resistance, carnivore, herbivore livestock, omnivore, urban wildlife, wildlife.

Antimicrobial resistance (AMR) is a serious worldwide issue in both human and animal medicine. Livestock and fish in aquaculture are often exposed to antimicrobials (Marshall and Levy 2011), and wild animals may acquire antimicrobial resistant bacteria and AMR genes via improperly disposed antimicrobial chemicals and via environments contaminated by feces and animal remains (Dolejska et al. 2007). Previous studies have shown that AMR bacteria are common in wildlife that live in close contact with humans and livestock, but it is uncertain whether more remote wildlife harbor AMR bacteria (Arnold et al. 2016).

A preliminary study conducted on AMR bacteria in wild animals (Don Bamunusinghage et al. 2019) in the eastern region of Sri Lanka indicated the presence of AMR, including multidrug-resistant (MDR) isolates, in *E. coli*, mainly in carnivores and omnivores: Jungle cat (*Felis chaus*), Serpent Eagle (*Spilornis cheela*), and Jungle Fowl (*Gallus lafayetti*). Our study aimed to identify the prevalence of AMR *E. coli* from three different animal groups, wildlife (WL), urban wildlife (UWL), and livestock in the eastern wildlife health region (EWHR) of Sri Lanka (Supplementary Material Table S1). An additional objective was to compare the presence of AMR in *E. coli* from carnivores, omnivores, and herbivores.

Fecal samples were collected from 47 wild animals, 54 urban wild animals, and 64 livestock including cattle, buffalo, pigs, ducks, and chicken (Supplementary Material Table S2) during the period from August 2018 to January 2019. The wild animals inhabited national parks and sanctuaries in the EWHR; the urban wild animals lived close to urban areas and the livestock lived close to national parks and sanctuaries. Fecal samples were collected onto sterile swabs either by swabbing voided feces from observed animals or directly from the rectum or cloaca. At the laboratory, samples were enriched in buffered peptone water at 37 C for 18–24 h and then plated onto MacConkey agar. Presumptive *E. coli* colonies on MacConkey agar plates were

TABLE 1. Antimicrobial resistance of the *Escherichia coli* isolated from wildlife (WL), urban wildlife (UWL), and livestock from the eastern region of Sri Lanka during the period of August 2018 to January 2019. The Fisher exact test in Prism was used.

Animal category	No. samples	Samples positive for *E. coli*, *n*/total (%)	*E. coli* isolates resistant to at least one antimicrobial, *n*/total (%)	Multidrug-resistant isolates, *n*/total (%)
WL	47	36/47 (76.6)	19/36 (52.7)[a]	08/36 (22.2)
UWL	54	45/54 (83.3)	9/45 (20.0)[b]	04/45 (8.8)
Livestock	64	48/64 (75.0)	25/48 (52.0)[c]	12/48 (25.0)
Total	165	129/165 (78.1)	53/129 (41.0)	24/129 (18.6)

[a] WL vs. UWL, $P=0.0025$.

[b] WL vs. livestock, $P>0.9999$.

[c] UWL vs. livestock, $P=0.0023$.

selected and subjected to biochemical tests for confirmation as *E. coli* (Quinn et al. 2011; Don Bamunusinghage et al. 2019). The AMR profiles of the isolated *E. coli* were detected using a standardized disk diffusion method (CLSI 2013) for 12 antimicrobials. The breakpoints provided by the CLSI (2013) were used for designation of isolates as resistant (R), intermediately susceptible (IS), or susceptible (S). For subsequent data analysis, the isolates with an I result were grouped with the isolates that gave an R result, and defined as resistant. Multidrug-resistant isolates were identified based on the definition of MDR as bacteria that are resistant to three or more classes of antimicrobial agents. We used *E. coli* American Type Culture Collection 25922 as the quality control strain in all procedures including the disk diffusion method. Results were analyzed using Prism (GraphPad 2021).

The proportion of *E. coli*-positive samples isolated from voided feces versus those from the rectum or cloaca did not significantly differ when analysed by the Fisher exact test (P values for voided feces versus rectal or cloacal samples were 0.64, 0.89, and 0.8, respectively, in WL, UWL, and livestock). From the total of 165 samples, 129 (78.1%) were positive for *E. coli*: 36/47 (77%) WL samples, 45/54 (83%) UWL samples, and 48/64 (75%) livestock. Of the 129 *E. coli*-positive samples, 25 came from carnivores, 69 from omnivores, and 35 from herbivores.

From the 36 WL isolates, 19 (53%) were resistant to at least one antimicrobial agent, and resistance was most frequently observed against ampicillin (49%) followed by tetracycline (28%), streptomycin (25%), and trimethoprim-sulfamethoxazole (18%); eight of the 19 (22%) were MDR. From the 45 UWL isolates, nine (20%) were resistant to at least one antimicrobial, and resistance was most frequently observed against streptomycin (32%), followed by ampicillin (24%) and nalidixic acid (8%); four of the nine isolates (9%) were MDR. From the 48 livestock isolates, 25 (52%) were resistant to at least one antimicrobial agent, and resistance was most frequently observed against ampicillin (49%), followed by nalidixic acid (34%), streptomycin (26%), trimethoprim-sulfamethoxazole (26%), and tetracycline (23%); 12 (25%) were MDR (Table 1 and Fig. 1). Interestingly, compared with WL and livestock groups, UWL showed significantly fewer resistant isolates (Table 1).

The frequency of AMR in *E. coli* isolates from carnivores, omnivores, and herbivores (Table 2) was analyzed using the Fisher exact test and by calculating the odds ratio. Carnivores showed a significantly higher chance of carrying *E. coli* with AMR compared with herbivores (odds ratio [OR] 8.4792; $P<0.05$; 95% confidence interval [CI], 5.0–74.6) or to omnivores (OR 21.19; $P<0.05$; CI, 2.3–30.9). There was no statistically significant difference in the frequency of *E. coli* with AMR between omnivores and herbivores (OR 2.498; $P>0.05$; CI, 1.1–5.8).

According to our results, *E. coli* from WL and livestock had AMR toward a common set of

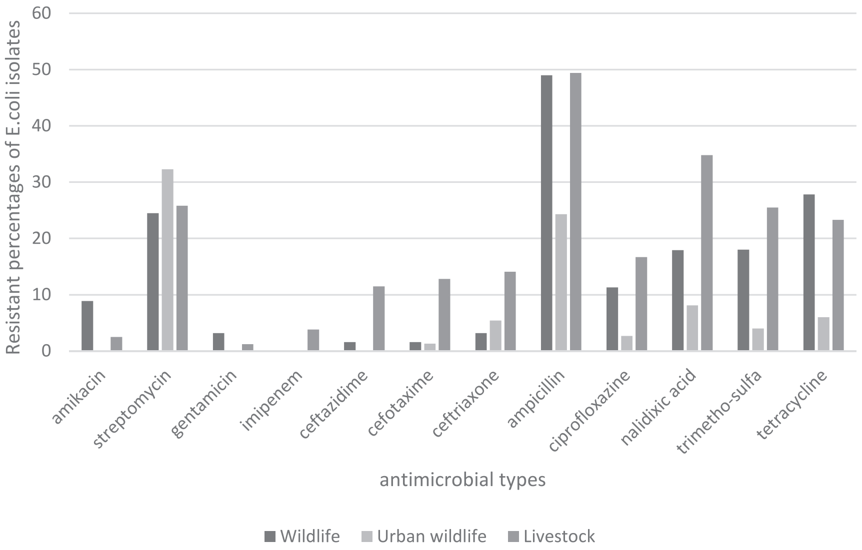

FIGURE 1. The percentages of *Escherichia coli* isolates from fecal samples of wildlife, urban wildlife, and livestock animals in the eastern region of Sri Lanka during 2019 showing resistance to 12 different antimicrobials. trimetho-sulfa=trimethoprim-sulfamethoxazole.

antimicrobials. This could be due to transmission of AMR bacteria from livestock to wild animals, as we have observed that they share common water resources. These AMR bacteria can contaminate soil and water systems and spread to wild animals. The use of antimicrobial growth promoters in livestock feed has been banned in Sri Lanka since 2018 (Priyankarage 2019) There is no evidence whether or not these regulations are implemented by the livestock sector. Our results indicate that AMR in bacteria is ongoing; it is necessary to monitor the use of antimicrobial growth promoters and other antibiotics in veterinary care.

TABLE 2. Differences of prevalence of antimicrobial resistance *Escherichia coli* isolated from carnivores, omnivores, and herbivores in the eastern region of Sri Lanka in 2019. The Fisher exact test in Prism was used.

Animal type based on feeding habit	*P*-value	Odds ratio	95% Confidence interval
Carnivore vs. omnivore	0.0003	8.4792	2.3206–30.9814
Carnivore vs. herbivore	<0.0001	21.19	5.032–74.60
Omnivore vs. herbivore	0.0560	2.498	1.056–5.769

In addition, our study included WL samples from animals in Kumana and Maduruoya national parks (NP) that are bordered by brackish water and freshwater tanks that are used for aquaculture. This suggests that AMR in aquatic animals may be another potential source for transmitting AMR to wildlife. Furthermore, every year during the month of August, there is a large cultural event in northern Sri Lanka in which thousands of people travel across the Kumana NP and temporally lodge in the park. Those types of human-wildlife interactions could lead to contamination of WL with human food waste and feces. Because this NP is famous for different kinds of migratory birds, AMR bacteria in the study population and associated environment may be widely transmitted to other regions of Sri Lanka and to other countries.

Interestingly and unexpectedly, compared with WL and livestock groups, UWL showed fewer resistant isolates. This group of UWL was located in the major cities of the eastern region of the Sri Lanka, Ampara, and Mahiyanganaya and lived within a 1-km radius of the cities. Without detailed studies to understand the feeding behaviors and food and water resources available to this UWL, it is difficult to further explain this unexpected finding.

We found that frequency of AMR in fecal *E. coli* isolates from carnivores was significantly higher than from omnivores and herbivores. This carnivore group includes raptors, fishing cats, and some water birds. Raptors and fishing cats often feed on small mammals, such as rats, which frequently consume animal and human food waste. Our results generally correlate with previous studies showing high AMR bacteria among wild birds such as raptors and gulls (Poeta et al. 2008; Guenther et al. 2010) and mammalian predators and omnivorous animals (Goncalves et al. 2013). Additionally, omnivorous animals frequently feed on carcasses and live in close proximity to humans and domestic animals, which may increase the risk of AMR transmission. Top predators may be more prone to acquire AMR bacteria due to their feeding habits and position in the food chain.

Our study confirms that AMR bacteria are widespread among WL, UWL, and livestock in the EWHR of Sri Lanka. The development of MDR *E. coli* within WL, UWL, and domestic animals is a threat to human health and welfare. More studies are required to determine the scale of this threat and to devise means to reduce the potentially harmful impact of widespread AMR bacteria.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/JWD-D-21-00048.

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