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FROM WHENCE THEY CAME—ANTIBIOTIC-RESISTANT *ESCHERICHIA COLI* IN AFRICAN WILDLIFE

Sarah Elizabeth Jobbins[1,2] and Kathleen Ann Alexander[1,2,3]

[1] Department of Fish and Wildlife Conservation, Virginia Polytechnic Institute and State University, Office 214, Laboratory 2102, 1981 Kraft Drive, Blacksburg, Virginia 24060, USA
[2] Centre for Conservation of African Resources: Animals, Communities and Land use (CARACAL), PO Box 570, Kasane, Botswana
[3] Corresponding author (email: kathyalx@vt.edu)

ABSTRACT: The emergence of antimicrobial resistance is arguably the most important threat to human and animal health. The impacts of antimicrobial use can reach far from the site of prescription and wildlife may serve as a conduit for the movement of resistance across landscapes, contributing to the spread of antimicrobial resistance within and between different reservoirs. We compared antimicrobial resistance and life history among wild and domestic species in Chobe, Botswana to explore key attributes and behaviors that may increase exposure and allow resistance to move between humans, animals, and ecosystems. Among 150 fecal samples evaluated from African animals, 41.3% contained *Escherichia coli* isolates that were resistant to one or two of 10 tested antibiotics, and 13.3% of isolates demonstrated multidrug resistance (three or more antibiotics). Resistance to each of the 10 tested antibiotics was detected among wildlife fecal samples. Resistance was widespread, but not ubiquitous, and isolates from wildlife demonstrated similar patterns of resistance to human *E. coli* from environmental and clinical sources in the study area. Multidrug resistance was significantly higher in carnivores, water-associated species, and species inhabiting urban areas, suggesting that life history may be key to understanding exposure patterns and transmission dynamics in heterogeneous landscapes.

Key words: Antimicrobial resistance, Botswana, disease ecology, emerging infectious disease, human–wildlife interface, life history, wildlife, zoonotic.

INTRODUCTION

Antimicrobial resistance is an emerging problem of global proportions, resulting in therapeutic failure and increased morbidity and mortality among affected individuals. Our antimicrobial arsenal is dwindling and resistance is on the rise, reflected in the resurgence of historic diseases such as extensively drug-resistant tuberculosis, *Clostridium difficile* diarrhea, and methicillin-resistant *Staphylococcus aureus*. In the US, more than two million cases of illness and at least 23,000 deaths occur each year as a result of infection with antimicrobial-resistant organisms (Centers for Disease Control and Prevention 2013). In developing countries where the burden of infectious disease is high, poor infection control (low immunization rates, hospital overcrowding), limited diagnostic capabilities, inconsistent prescribing behavior, and the availability of antibiotics over the counter likely all contribute to the emergence of resistance (reviewed by Laxminarayan and Heymann 2012).

Despite significant advances in our understanding of the molecular dynamics (Walsh 2000) and widespread occurrence of antimicrobial resistance across a diversity of hosts (Rolland et al. 1985; Goldberg et al. 2007; Rwego et al. 2008; Blackburn et al. 2010; Wheeler et al. 2012; Pesapane et al. 2013) and environments (Pruden et al. 2006), it remains unclear how the transmission of resistance occurs across these complex landscapes and host communities. This information is critical to prevention. Wildlife provides a unique opportunity to investigate landscape dynamics of antimicrobial resistance—each species occupies a particular niche and interacts with the environment in different and specific ways, dependent on key life-history strategy elements. In this respect, wildlife communities may act as sentinels for ecosystem health, providing clues to points where human and natural systems

are coupled and transmission of antimicrobial resistance occurs.

In the Chobe District, northern Botswana, humans and wildlife live in proximity, their spatial dynamics driven by strong seasonal regimes that influence the distribution of surface water resources (Alexander et al. 2012). Primary health care is available in the region; antibiotics can be acquired freely over the counter and resistance to many first-line antimicrobials is common (Rowe et al. 2010; Pesapane et al. 2013; Renuart et al. 2013). There are no commercial livestock or poultry production operations in the region. We have already demonstrated that significant antimicrobial resistance exists in *Escherichia coli* isolated from humans and banded mongoose (*Mungos mungo*) in Chobe (Pesapane et al. 2013). Here, we assess the extent of antimicrobial resistance among fecal *E. coli* isolates collected from a diversity of animal species, and explore the complex and potentially interdependent drivers that may shape microbial exchange across heterogeneous landscapes.

MATERIALS AND METHODS

Study site description

Botswana is a landlocked country in sub-Saharan Africa with a subtropical climate and highly variable rainfall. Our study site (Fig. 1) is located within the Chobe District in northern Botswana, an area of 20,800 km^2 that includes the Chobe National Park (a protected land area, managed to achieve long-term conservation of natural resources) and the adjacent villages of Kasane and Kazungula (unprotected urban/periurban areas). The Kwando–Chobe–Linyanti River system floods in the dry season (April–October), arising from rainfall in the Angolan highlands, and is the sole source of water to the area for much of the year. During the dry season, huge shifts in biomass occur, prompted by a scarcity of resources in the interior, concentrating humans and animals along the banks of the Chobe River.

Sample collection

Three technicians (an observer, a record keeper, and a Basarwa tracker) collected fecal samples between June and September 2011. We have used the same tracker over the last decade to reliably identify animal spoor and fecal samples to the species of origin and determine fecal age class (i.e., <24 h, >24 h, <1 wk, >1 wk). The observer and tracker were the same individuals throughout the sampling. Fifty-five stratified transect points, 100 m long, were placed at 500-m intervals along the Chobe River (Fig. 1), starting at the confluence of the Chobe and Zambezi rivers (transect 1: 17°47′39.9114″S, 25°15′38.5554″E) and extending 27.5 km upstream, into the Chobe National Park (transect 55: 17°49′55.4154″S, 25°2′53.0874″E). This section of the river has been the focus of a long-term water-quality project, work complementary to this study. Sampling commenced at transect 1 and continued upstream to transect 55, and any feces (wildlife or domestic animal) seen while traversing the transect line were identified to species, sampled, and the GPS location recorded. Using aseptic technique, approximately 5 g was taken from the center of the fecal ball using a sterile tongue depressor and collected in a sterile 50-mL conical tube. Feces that could not be reliably identified because of disruption and poor surface type (failed spoor or footprint detection), or were older than 24 h (as determined by the tracker), were excluded from the study. Fecal samples were transported under cooled conditions and stored at −20 C within 4 h of collection. A complete sampling (transects 1–55) took approximately 5 d, and sampling was repeated monthly for a total of three sampling exercises.

One gram of feces from each sample was homogenized by vortexing in 9 mL of buffered peptone water (BPW; Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) and serially diluted to $1{:}10^2$, $1{:}10^3$, and $1{:}10^4$ in BPW. We plated 100 μL of the $1{:}10^3$ and $1{:}10^4$ dilutions on two MacConkey agar plates (Thermo Fisher Scientific Inc., Lenexa, Kansas, USA) and incubated the plates at 37 C for 18 h. Plates were inspected for adequate growth (50–80 well-spaced colonies), and six individual colonies of pink to rose-red morphology were picked per sample and placed separately into tryptic soy broth (TSB; EMD Millipore, Billerica, Massachusetts, USA), following the methods of Goldberg et al. (2006) and Pesapane et al. (2013). We incubated TSB cultures at 37 C for 18 h and extracted DNA by heat/detergent lysis and ethanol precipitation.

The DNA extracts were screened for *E. coli* DNA by PCR, using genera-specific *malB*

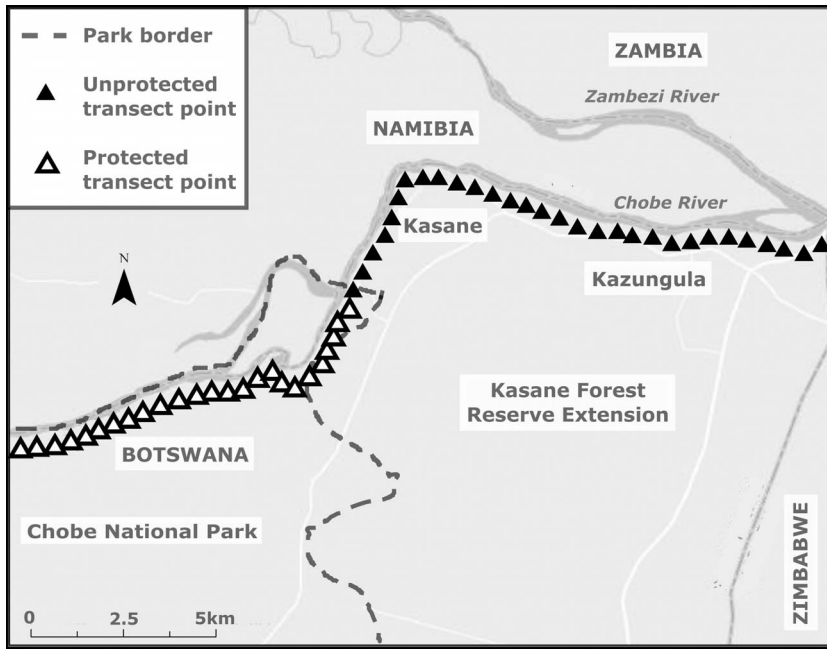

FIGURE 1. Fifty-five sampling transects were systematically identified for fecal surveys, running perpendicular to the Chobe River across animal density gradients, spanning both protected (Chobe National Park; open triangles) and unprotected (villages of Kasane and Kazungula; filled triangles) land areas in northern Botswana, 2011.

primers ECO-1 and ECO-2 (Wang et al. 1996). The PCR was conducted in a MyCycler™ thermocycler (Bio-Rad, Hercules, California, USA) in reactions of 25 μL containing 1.5 μM each primer, 1× Go Taq Master Mix (Promega, Madison, Wisconsin, USA), 0.3 μL of 10% dimethyl sulfoxide, and 1 μL of DNA (approximately 100 ng). Cycling conditions were set at 95 C (15 min), followed by 30 cycles of 95 C (30 s), 55 C (45 s), and 72 C (45 s), with final extension at 72 C (10 min). For quality assurance, positive (*E. coli* strain ATCC 25922 DNA) and negative (water) control assays were run with each round of PCR. Isolates confirmed as *E. coli* were tested for sensitivity to 10 antimicrobials (10 μg of ampicillin, 30 μg of chloramphenicol, 5 μg of ciprofloxacin, 30 μg of doxycycline, 10 μg of gentamicin, 30 μg of neomycin, 10 μg of streptomycin, 30 μg of tetracycline, 25 μg of trimethoprim-sulfamethoxazole, and 30 μg of Ceftiofur; Thermo Fisher Scientific) using the Kirby-Bauer disk diffusion method (Bauer et al. 1966), following the protocols and quality controls indicated by the Clinical and Laboratory Standards Institute (CLSI 2009). To remain conservative in our estimates of resistance, isolates exhibiting intermediate zones of inhibition were interpreted as sensitive.

Results were compared with resistance data for *E. coli* isolated from 193 human clinical specimens (blood, urine, stool, and vaginal swabs; one isolate per specimen) evaluated in the laboratory of the Kasane Primary Hospital, Kasane, Botswana, between July 2007 and July 2011. The laboratory serves the entire human population living in the Chobe District, and utilizes the same technique (Kirby-Bauer disk diffusion) to evaluate antimicrobial sensitivity. In contrast to isolates from animal feces, human isolates demonstrating intermediate zones of inhibition are classified as resistant in this clinical setting, to reduce therapeutic failure related to antibiotic resistance. Samples originated in both healthy (employment health certification) and clinically ill patients, providing useful insight into the prevalence and nature of antibiotic resistance that may occur in the local human population.

We also compared our findings with 77 *E. coli* isolates from 12 environmental sources of human fecal waste (bush latrines, sewage sludge, evaporation ponds, and wastewater leakage) sampled in the study area between June and September 2010 (Pesapane et al. 2013). These samples were processed as described above; however, 15 *E. coli* colonies were selected per sample (instead of six). The

number of human environmental samples was low compared with human clinical and animal fecal samples. However, this was a reflection of fewer environmental sources of human fecal waste and their difficulty in being located before being destroyed, consumed by animals, or older than 24 h and desiccated.

Data analysis

We categorized species on the basis of interactions with the environment (diet, association with water, and land-use). Diet was defined as primarily carnivorous (crocodile, *Crocodylus niloticus*; spotted hyena, *Crocuta crocuta*; leopard, *Panthera pardus*; otter, *Aonyx capensis*), herbivorous (Cape buffalo, *Syncerus caffer*; bushbuck, *Tragelaphus scriptus*; domestic cattle, *Bos primigenius*; elephant, *Loxodonta africana*; giraffe, *Giraffa camelopardalis*; hippopotamus, *Hippopotamus amphibious*; impala, *Aepyceros melampus*; greater kudu, *Tragelaphus strepsciseros*; sable, *Hippotragus niger*; waterbuck, *Kobus ellipsiprymnus*), or omnivorous (Chacma baboon, *Papio ursinus*; guineafowl, *Numida meleagris*; banded mongoose; vervet monkey, *Chlorocebus pygerythrus*; warthog, *Phacochoerus africanus*). Water-associated species were defined as those with an aquatic or semiaquatic life-history strategy, or who graze primarily on water-inundated floodplains (crocodile, hippopotamus, otter, and waterbuck). Land use was defined as protected (Chobe National Park) or unprotected (urban/periurban villages of Kasane and Kazungula) on the basis of the location where the fecal sample was collected. Samples reflected the density of species in the region, with some guilds (e.g., herbivores) naturally occurring in higher numbers than others (e.g., carnivores). To avoid potential bias from uneven numbers of isolates per sample, statistical comparisons were only conducted at the fecal sample level. A fecal sample was defined as resistant if at least one of its constituent isolates was resistant to one or more antibiotics, and multidrug resistant if at least one of its isolates was resistant to three or more antibiotics. A fecal sample containing two resistant isolates was still only classified as resistant. Statistical analyses were conducted using the binom and stats packages in R version 3.0.0, www.R-project.org, and Bonferroni-adjusted alpha values were applied where necessary to correct for multiple comparisons.

This study was conducted under permit from the Botswana Ministry of Environment, Wildlife, and Tourism (EWT 8/36/4 XXVI [24]), and the Institutional Animal Care and Use Committee at Virginia Tech (FIW 13-164). The study did not involve capture or handling of any live animals.

RESULTS

We evaluated 900 isolates from 150 fecal samples of wildlife (18 species) and domestic cattle. Of these, 48.9% ($n=900$; 95% confidence interval [CI] 45.6–52.2%) were confirmed as *E. coli* and subjected to antibiotic susceptibility testing (Table 1). No antimicrobial resistance was detected in feces from domestic cattle. However, 43.4% ($n=143$; 95% CI 35.1–51.9%) of wildlife fecal samples harbored *E. coli* resistant to one or more antibiotics (Table 1). Multidrug-resistant isolates were identified in 13.9% (95% CI 8.8-20.8%) of wildlife fecal samples (Table 1). Resistance to each of the 10 tested antibiotics was detected among wildlife fecal samples.

Resistance was high among human clinical specimens ($n=193$) from the local primary hospital; 94.3% of specimens (95% CI 90.0–97.1%) were resistant to one or more antibiotics and 68.9% (95% CI 61.9–75.4%) were multidrug resistant. The *E. coli* from wildlife, human clinical, and environmental samples were resistant to a similar spectrum of antibiotics (most commonly ampicillin, doxycycline, streptomycin, tetracycline, or trimethoprim-sulfamethoxazole), albeit at a lower prevalence in wildlife than in humans (Fig. 2).

Prevalence of antimicrobial resistance was influenced by life-history strategy of the animal host species (Table 2). There was a significant difference in both the overall prevalence of resistance ($P=0.006$) and in multidrug resistance ($P=0.001$) among carnivores, omnivores, and herbivores (Table 2). Resistance was more prevalent in carnivores, followed by omnivores, and herbivores. Multidrug resistance was significantly more prevalent in water-associated than nonwater-associated species ($P=0.006$; Table 2). Similarly, multidrug resistance was significantly more prevalent in wildlife from the unprotected

TABLE 1. Prevalence of antimicrobial resistance in feces from African animals sampled in northern Botswana in 2011 (based on isolated *Escherichia coli*).

Species	N[a]	Resistant[b]	Multidrug resistant (MDR)[b]
African elephant (*Loxodonta africana*)	48 (122)	41.7 (20/48; 27.6–56.8)	14.6 (7/48; 6.1–27.8)
Banded mongoose (*Mungos mungo*)	2 (5)	100.0 (2/2; 15.8–100.0)	0.0 (0/2; 0.0–84.2)
Bushbuck (*Tragelaphus scriptus*)	1 (1)	100.0 (1/1; 25.0–100.0)	0.0 (0/1; 0.0–97.5)
Cape buffalo (*Syncerus caffer*)	8 (30)	25.0 (2/8; 3.2–65.1)	0.0 (0/8; 0.0–36.9)
Chacma baboon (*Papio ursinus*)	18 (54)	66.7 (12/18; 40.1–86.7)	22.2 (4/18; 6.4–47.6)
Crocodile (*Crocodylus niloticus*)	2 (12)	50.0 (1/2; 1.3–98.7)	0.0 (0/2; 0.0–84.2)
Domestic cattle (*Bos primigenius*)	7 (20)	0.0 (0/7; 0.0–53.1)	0.0 (0/7; 0.0–53.1)
Giraffe (*Giraffa camelopardalis*)	2 (8)	50.0 (1/2; 1.3–98.7)	0.0 (0/2; 0.0–84.2)
Greater kudu (*Tragelaphus strepsciseros*)	3 (9)	0.0 (0/3; 0.0–70.8)	0.0 (0/3; 0.0–70.8)
Guineafowl (*Numida meleagris*)	1 (4)	0.0 (0/1; 0.0–97.5)	0.0 (0/1; 0.0–97.5)
Hippopotamus (*Hippopotamus amphibius*)	6 (16)	50.0 (3/6; 11.8–88.2)	16.7 (1/6; 0.4–64.1)
Impala (*Aepyceros melampus*)	18 (49)	27.8 (5/18; 9.7–53.5)	0.0 (0/18; 0.0–18.5)
Leopard (*Panthera pardus pardus*)	1 (6)	100.0 (1/1; 25.0–100.0)	0.0 (0/1; 0.0–97.5)
Otter (*Aonyx capensis*)	4 (14)	100.0 (4/4; 39.8–100.0)	100.0 (4/4; 39.8–100.0)
Sable (*Hippotragus niger*)	2 (4)	0.0 (0/2; 0.0–84.2)	0.0 (0/2; 0.0–84.2)
Spotted hyena (*Crocuta crocuta*)	1 (5)	100.0 (1/1; 25.0–100.0)	100.0 (1/1; 25.0–100.0)
Vervet monkey (*Chlorocebus pygerythrus*)	1 (1)	0.0 (0/1; 0.0–97.5)	0.0 (0/1; 0.0–97.5)
Warthog (*Phacochoerus africanus*)	21 (62)	33.3 (7/21; 14.6–57.0)	9.5 (2/21; 1.2–30.4)
Waterbuck (*Kobus ellipsiprymnus*)	4 (18)	50.0 (2/4; 6.8–93.2)	25.0 (1/4; 0.6–80.6)
Total	150 (440)	41.3 (62/150; 33.4–49.7)	13.3 (20/150; 8.3–19.8)

[a] N = number of fecal samples (number of isolates) evaluated.

[b] Prevalence (%) of fecal samples resistant or MDR (n/N; exact binomial 95% confidence interval); resistant fecal samples contained *E. coli* isolate(s) that were resistant to one or more antibiotics; MDR fecal samples contained *E. coli* isolate(s) that were resistant to three or more antibiotics.

(urban and periurban) than the protected (Chobe National Park) areas ($P=0.013$; Table 2).

DISCUSSION

This study is one of few to examine antimicrobial resistance in a broad range of hosts (both in absolute numbers and breadth of species) in their natural environment (Gordon and Cowling 2003; Benavides et al. 2012; Lescat et al. 2013) and the first to use a comparative life-history study design to evaluate potential mechanisms of exposure across different land-use areas.

Resistance was widespread but not ubiquitous among sampled animals, and divergent life-history attributes—diet, water association, and tolerance for humans/urban and periurban occurrence—were associated with increased accumulation of antimicrobial resistance in the animal species evaluated.

Apex predators act as important ecosystem sentinels (or "condition indicators"), as they are at the top of the food chain (Sergio et al. 2008). In a compromised ecosystem, trophic accumulation of pollutants (Kannan et al. 2004), pesticides (Newton 1979), and fecal coliforms (Blackburn et al. 2010) can occur, threatening a guild that already exists at lower densities. In this investigation, diet appears to be an important factor in the accumulation of resistance, where resistance was higher in carnivores (leopard, crocodile, otter, and hyena), followed by omnivores (baboon, banded mongoose, vervet monkey, and warthog) and last, herbivores (all others). These findings suggest that antimicrobial resistance may follow a similar pattern of trophic accumulation, as suggested elsewhere in red foxes (*Vulpes vulpes* [Grobbel et al. 2012]) and birds of prey (Radhouani et al. 2014).

Water can be an important vehicle for distributing fecal contaminants across the

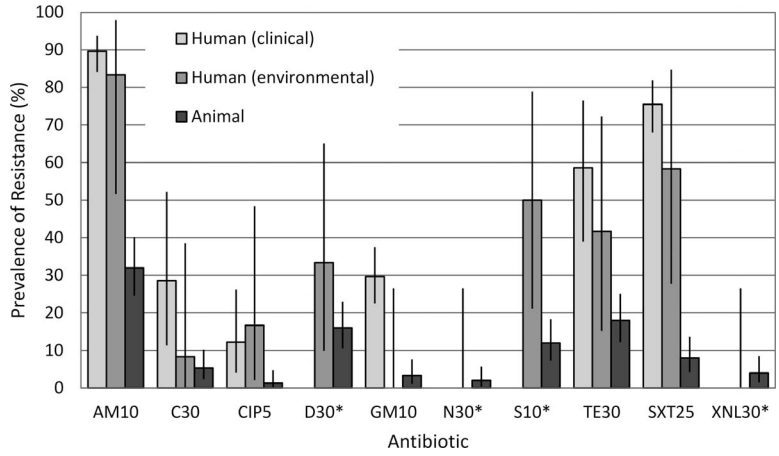

FIGURE 2. Prevalence (%) of antibiotic resistance (based on isolated *Escherichia coli*) among animal feces ($n=150$), environmental sources of human waste ($n=12$), and human clinical specimens ($n=193$) from Chobe, Botswana subjected to routine laboratory microorganism susceptibility assessments at the Kasane Primary Hospital, Kasane, Botswana. Error bars indicate exact binomial 95% confidence intervals (multiplied by 100 to produce scale bars representing percentages). Asterisks (*) indicate those antibiotics not evaluated in human clinical samples. The antibiotic panel was comprised of ampicillin, AM10; chloramphenicol, C30; ciprofloxacin, CIP5; doxycycline, D30; gentamicin, GM10; neomycin, N30; streptomycin, S10; tetracycline, TE30; trimethoprim-sulfamethoxazole, SXT25; and Ceftiofur, XNL30.

TABLE 2. Prevalence of antimicrobial resistance in feces from African animals sampled in northern Botswana in 2011 (based on isolated *Escherichia coli*), stratified by key life history trait.

Life history trait	N[a]	Resistant[b]	P[c]	Multidrug resistant (MDR)[b]	P[c]
Diet[d]			0.006		0.001
Carnivore	8 (37)	87.5 (7/8; 47.3–99.7)		62.5 (5/8; 24.5–91.5)	
Omnivore	43 (126)	48.8 (21/43; 33.3–64.5)		14.0 (6/43; 5.3–27.9)	
Herbivore	99 (277)	34.3 (34/99; 25.1–44.6)		9.1 (9/99; 4.2–16.6)	
Water[e]			0.051		0.006
Water-associated	15 (60)	66.7 (10/15; 38.4–88.2)		40.0 (6/15; 16.3–67.7)	
Not water-associated	135 (380)	38.5 (52/135; 30.3–47.3)		10.4 (14/135; 5.8–16.8)	
Land use[f]			0.85		0.013
Protected	111 (319)	40.5 (45/111; 31.3–50.3)		9.0 (10/111; 4.4–15.9)	
Unprotected	39 (121)	43.6 (17/39; 27.8–60.4)		25.6 (10/39; 13.0–42.1)	

[a] N = number of fecal samples (number of isolates) evaluated.

[b] Prevalence (%) of fecal samples resistant or MDR (n/N; exact binomial 95% confidence interval).

[c] Fisher's exact P values.

[d] Diet was defined as carnivorous (crocodile, leopard, lion, and otter), omnivorous (baboon, guineafowl, banded mongoose, vervet monkey, and warthog), or herbivorous (buffalo, bushbuck, domestic cattle, elephant, giraffe, hippopotamus, impala, greater kudu, sable, and waterbuck).

[e] Water use was defined as water-associated (crocodile, hippopotamus, otter, and waterbuck) or nonwater associated (elephant, mongoose, bushbuck, buffalo, baboon, cattle, giraffe, kudu, guineafowl, impala, leopard, sable, hyena, vervet monkey, and warthog).

[f] Land use was defined by the location of the fecal sample in protected (Chobe National Park) or unprotected (urban/periurban surrounding villages) land use areas; resistant fecal samples contained *E. coli* isolate(s) that were resistant to one or more antibiotics; MDR fecal samples contained *E. coli* isolate(s) that were resistant to three or more antibiotics.

landscape, facilitating indirect contact between humans and animals (Radhouani et al. 2014). In this manner, water can act as a powerful exposure medium for the introduction of antimicrobial resistance into naïve populations (Mariano et al. 2009). Human fecal material can enter waterways with stormwater runoff, sewage system breaks, or flooding of septic tanks (McCarthy et al. 2004), as well as direct defecation and pollution from livestock and wildlife when animals congregate around water resources. In this investigation, water-associated species (those living or foraging within the river system) harbored higher levels of antibiotic resistance than nonwater-associated species. Consumption of water alone was not associated with resistance, however, as several water-dependent species (consumption of water due to physiological requirements) had little or no evidence of exposure (e.g., impala, buffalo, cattle, and sable). Rather, those species with aquatic or semiaquatic life-history strategies (crocodile, hippopotamus, otter, and waterbuck) harbored greater levels of multidrug-resistant *E. coli.* These findings suggest that—among other possible factors—the duration of exposure and consumption of water-inundated vegetation and sediment might be key to exposure and transmission of resistant microbes. Indeed, among avian species, waterfowl have been associated with increased carriage of extended-spectrum beta-lactamase–producing *E. coli* and vancomycin-resistant enterococci (reviewed by Radhouani et al. 2014). In addition to the gastrointestinal system of warm-blooded animals, water and sediment can also be important habitats of *E. coli* (Savageau 1983).

Accumulation of multidrug resistance in wildlife is often attributed to anthropogenic influence (Rolland et al. 1985; Skurnik et al. 2006; Goldberg et al. 2007; Rwego et al. 2008; Allen et al. 2010; Wheeler et al. 2012; Lescat et al. 2013). In this study, multidrug resistance was significantly more common in the unprotected urban/periurban area where most human activity occurs (Table 2). Supporting this, human and wildlife *E. coli* isolates from the same ecosystem demonstrated similar patterns of resistance to antimicrobials (Fig. 2). Furthermore, antibiotic resistance was prevalent in several species with a tendency to associate with humans, including baboon, banded mongoose, and warthog (i.e., peridomestic species). This is consistent with other studies where animals foraging in human settlements and refuse dumps harbored significant amounts of antibiotic-resistant bacteria (Rolland et al. 1985; Pesapane et al. 2013). More importantly, although these species are coupled with humans through waste utilization, individuals can move across unprotected and protected land areas, connecting human populations with a myriad of other wildlife species across the ecosystem and providing a mechanism by which antibiotic-resistant microbes may be distributed throughout the landscape.

In the absence of more specific molecular investigations, we cannot exclude the possibility that the source of the antibiotic resistance in some cases was not human. The development of antimicrobial resistance is a complex phenomenon, arising by direct selection from clinical or agricultural antibiotic use, independent selection by naturally occurring heavy metals and antibiotics, or via the transfer of naturally occurring resistance elements from environmental bacteria (Allen et al. 2010; Wellington et al. 2013). The presence of antimicrobial resistance in wildlife also does not necessarily imply direct transmission from humans (Benavides et al. 2012), and horizontal gene movement readily occurs in areas of high microbial density (Salyers et al. 2004; Schlüter et al. 2007), allowing resistance determinants to be exchanged between microorganisms. Molecular evaluations, such as repetitive-element PCR (Goldberg et al. 2006), will be essential to determine the dynamics of transmission in this system, and are underway in our laboratory.

The presence of antibiotic-resistant *E. coli* in this system is of grave concern because of the potential of wildlife to contribute to movement of resistance through the system (Guenther et al. 2011; Radhouani et al. 2014). Botswana has one of the highest rates of human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) in the world (Piot et al. 2001), and this epidemic has created a population highly vulnerable to disease. Indeed, communicable diseases (including malaria, HIV/AIDS, and tuberculosis) are responsible for 45% of deaths each year in Botswana (World Health Organization Regional Office for Africa [WHO-AFRO] 2014). Alarmingly, we demonstrated widespread resistance in wildlife to several first-line antimicrobials used in human medicine—ampicillin, doxycycline, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole (commonly known as cotrimoxazole). Doxycycline is a common antimalarial drug used by visitors to Africa (Lobel et al. 2001), cotrimoxazole is administered widely for prophylaxis against opportunistic infections in HIV patients (WHO 2006), and streptomycin is a first-line antituberculosis drug (Shi et al. 2007). Resistance to these front-line antimicrobials can necessitate implementation of second- and third-line antimicrobials, which—in developing countries such as Botswana—may result in increased morbidity and mortality because of prohibitive costs or lack of access to these drugs.

In this investigation, resistance was widespread but not ubiquitous across species. For example, no resistance was detected in cattle, guinea fowl, kudu, sable, or vervet monkey. Rather, key life-history traits appeared to be associated with exposure, revealing potentially important coupling points for exposure and transmission of antimicrobial-resistant *E. coli* among humans, wildlife, and the environment. Water clearly plays a focal but complex role in transmission of resistance elements, and diet may increase the risk of exposure to antimicrobial-resistant organisms through trophic accumulation. Capitalizing on their role as potential sentinels of aquatic and terrestrial ecosystems, long-term monitoring of antimicrobial resistance among apex predators, water-, and human-associated species may support improved surveillance, detection, and control of the spread of antimicrobial resistance across the landscape. This novel approach may be applied to other ecosystems, facilitating early detection of antimicrobial resistance epidemics.

As we lose our pharmacological arsenal to fight infectious diseases, emerging, re-emerging, and persistently occurring pathogens will find new windows of opportunity to invade, with dire consequences for public health. The use of sentinel wildlife as ecosystem indicators and identification of points of microbial connectivity will enable more focused surveillance and management of the antimicrobial resistance crises.

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