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Original Contribution

Antibiotic Resistance of *Escherichia coli* from Humans and Black Rhinoceroses in Kenya

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Abstract: Upsurge of antibiotic resistance in wildlife poses unprecedented threat to wildlife conservation. Surveillance of antibiotic resistance at the human–wildlife interface is therefore needed. We evaluated differences in antibiotic resistance of *Escherichia coli* isolates from human and the endangered black rhinoceros in Lambwe Valley, Kenya. We used standard microbiological techniques to carry out susceptibility assays using eight antibiotics of clinical and veterinary importance. Standard PCR method was used to characterize antibiotic resistance genes. There was no difference in resistance between *E. coli* isolates from human and those from rhinoceros ($U = 25$, $p = 0.462$). However, higher resistance in isolates from humans was noted for cotrimoxazole ($p = 0.000$, OR = 0.101), ceftriaxone ($p = 0.005$, OR = 0.113) and amoxicillin/clavulanic acid ($p = 0.017$, OR = 0.258), whereas isolates from rhinoceros showed higher gentamicin resistance ($p = 0.001$, OR = 10.154). Multi-drug resistance phenotype was 69.0% in humans and 43.3% in rhinoceros. Isolates from both species contained bla_{TEM}, *tetA*, *tetB, dfrA1* and *sul1* genes. Resistance profiles in the two species suggest potential for cross-transfer of resistance genes or exposure to comparable selective pressure and call for a multi-sectorial action plan on surveillance of antibiotic resistance at the human–wildlife interface. Genome-wide studies are needed to explicate the direction of transfer of genes that confer antibiotic resistance at the human–wildlife interface.

Keywords: Antibacterial resistance, *Escherichia coli*, Zoonotic, Multi-drug resistance, Black rhinoceros, Kenya

Introduction

Although antibiotic agents have been in use for decades, the emergence of a wide range of pathogens that are resistant to available antibiotics poses a major challenge to public health (WHO 2014). Global public health threat of antibiotic resistance is projected to increase in light of increasing antibiotic consumption (Klein et al. 2018). Between 2000 and 2015, the global consumption of antibiotics increased from 21.1 to 34.4 billion defined daily doses with much of the increase occurring in low- and middle-income countries (Klein et al. 2018). Evidence suggests that Africa shares the global burden of antibiotic resistance (Mbaga et al. 2010; Lim et al. 2016). In Kenya, for example, clinical studies point to high levels of resistance to a wide range of antibiotics such as ampicillin, gentamicin, tetracycline, cotrimoxazole, chloramphenicol, ciprofloxacin and erythromycin (Sang et al. 2012; Kipkorir et al. 2016).

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Efforts by the WHO and various antibiotic stewardship programs that aim to arrest the spread of antibiotic resistance are laudable (Macdougall and Polk 2005; WHO 2015). For instance, the WHO global action plan emphasizes the need for understanding both the development and spread of genes that confer antibiotic resistance in the complex network of feedbacks in the Earth's critical zone (Zhu et al. 2018). Although not explicitly spelled out in the WHO global action plan, the interface between human and wildlife landscapes and associated interactions is an important link in such networks. Indeed, antibiotic resistant bacteria are increasingly reported in wildlife (Silva et al. 2010; Pesapane et al. 2013; Foti et al. 2018; Swift et al. 2019).

Interactions between humans and wildlife including conservation measures such as translocation employed in recovery of species in peril bring such species under increased contact with humans. Increased contact with humans provides opportunities for cross-species transfer of genes that confer antibiotic resistance. In addition, conservation of critically endangered species may be imperiled by environmental sources of antibiotics (Zhu et al. 2018). In particular, water and soil may be major transmission routes where antimicrobial resistance genes exist due to production of antibiotics by some bacteria and fungi as reviewed by Vittecoq et al. (2016). A first step in understanding patterns of antibiotic resistance at the human–wildlife interface is to investigate profiles of antibiotic resistance and associated genes between humans and wildlife. To this end, commensal bacteria such as *Escherichia coli* are excellent candidates for monitoring antibiotic resistance in a population. According to Savageau (1983), *E. coli* spends approximately half its life cycle in the external environment such that substrates such as soil, surface or ground water contaminated with resistant *E. coli* and its genes may constitute a reservoir for their dissemination.

In this study, we investigated antibiotic resistance profiles and genes in isolates of a model microbe, *E. coli,* from human and those from a population of the critically endangered black rhinoceros *(Diceros bicornis michaeli)* reintroduced in Ruma National Park, Kenya, so as to understand the status of antibiotic resistance between the two species. Specifically, we aimed to determine differences in antibiotic resistance patterns against eight commonly used antibiotics and to characterize antibiotic resistance genes in *E. coli* isolates from humans and black rhinoceros. We targeted antibiotics that are commonly used both for clinical and veterinary management of bacterial infections: ampicillin, gentamicin, tetracycline, cotrimoxazole, chloramphenicol, ceftriaxone, amoxicillin/clavulanic acid, and erythromycin.

Materials and Methods

This was a cross-sectional study which involved recovery of *E. coli* isolates from human population living in close proximity to Ruma National Park and the reintroduced black rhinoceros population within the park. In 2011–2012, the Kenya Wildlife Service reintroduced 21 black rhinoceros from two private ranches, Mugie and Solio, to Ruma National Park (RNP). The reintroduction followed a near 50-year absence of rhinoceros from RNP and was meant to increase the tourism appeal of the park. There are presently about 20 black rhinoceros in the park. The park is also home to other large mammals including Roan antelope (*Hippotragus equinus*), Rothschild giraffe (*Giraffa camelopardalis rothschildi*), impala (Aepyceros melampus), bush buck (*Tragelaphus scriptus*), and white rhinoceros (*Ceratotherium simum simum*). Water supply to the park is primarily by the Lambwe River, which runs the whole length of the park. There is a high potential that the river conveys any anthropogenic wastes through the park. However, we note that agricultural practices in areas that neighbor the park are mostly small scale and there are not any large manufacturing plants or wastewater treatment plants, which are known hotspots for antibiotic resistance, in close proximity to the park.

Samples were collected between July and December 2017. Authorization to conduct the study in the park was obtained from the Kenya Wildlife Service: permit reference number KWS/BRM/5001. Ethical clearance was obtained from Maseno University Ethical Committee Reference Number MSU/DRPI/MUERC/00420/17. Informed consent was obtained from all participants included in the study.

Sample collection Fecal sample collection was conducted following Carroll et al. (2015), with minor modification. Fresh fecal samples were taken aseptically and inoculated in Cary-Blair media (Himedia pvt Ltd. Mumbai, India) in screw-capped universal bottles. Samples were then transferred in ice cool box at 8°C from the field to the central storage facility and stored for 4 days at 4°C until microbiological analysis.

Isolation of E. coli Isolation and identification of *E. coli* was performed according to protocol by Morello et al. (2003), where the transportation media was gently agitated

and aliquots sub-cultured in an enrichment media, tryptone phosphate broth (Himedia pvt Ltd. Mumbai, India), and incubated at 37°C for 20 h. The isolates were then sub-cultured in enrichment broth and gently agitated. Aliquot of the homogenate was streaked on Eosin Methylene Blue (EMB) agar (Himedia pvt Ltd. Mumbai, India) and incubated at 37°C for 24 h. Colony color and morphology was then determined by visualization using a hand lens. Colonies of typical *E. coli* were sub-cultured on Triple Sugar Iron (TSI) media (Himedia pvt Ltd. Mumbai, India) in caped tubes and incubated at 37°C for 48 h. The isolates from TSI showing both acidic slant and butt were sub-cultured on motility–indole–lysine medium (MIL) (Himedia pvt Ltd. Mumbai, India) and in methyl red and Voges-Proskauer *medium* (Himedia pvt Ltd. Mumbai, India) for biochemical differentiation and identification of *E. coli*.

Antibiotic susceptibility assay Antibacterial susceptibility test was performed following the protocol by Kirby-Bauer disk diffusion method (Bauer et al. 1966). Antibacterial susceptibility breakpoints were interpreted according to recommended standards by the Clinical and Laboratory Standard Institute (CLSI. 2016). The following antibacterial agents were considered in this study: ampicillin (10 mcg), gentamicin (10 mcg), tetracycline (30mcg), cotrimoxazole (25 mcg), chloramphenicol (30 mcg), ceftriaxone (30 mcg), amoxicillin/clavulanic acid (30 mcg) and erythromycin (15 mcg). In this case, *E. coli* cultures were swabbed onto a Mueller-Hinton agar *plate* (Himedia pvt Ltd. Mumbai, India) to form a bacteria lawn. Antibacterial disks (Himedia pvt Ltd. Mumbai, India) were applied using sterile forceps onto the culture plates. The culture plates were dried on working bench for 5 min after which they were incubated at 37°C for 24 h (Bauer et al. 1966). *Escherichia coli* ATCC 25922 was used as a control for potency of antibiotic agent disks.

DNA extraction and amplification DNA was extracted from *E. coli* isolates preserved at 4°C in *Soybean* Casein Digest Medium (Himedia pvt Ltd. Mumbai, India) with 15% glycerol using DNeasy purification kit (QIAGEN). Amplification of bla_{TEM}, *tetA*and *tetB*, *Sul1* and *dfrA1* genes was performed in Rotor-Gene PCR cycler (QIAGEN) using published primers (Table 1) in a final reaction volume of 50 µl. Initial denaturing at 94°C for 5 min was followed by 30 cycles at 94°C for 1 min, annealing at the respective temperatures for 45 s, elongation at 72°C for 2 min and final extension 72°C for 10 min then at 4°C until visualization. Amplicons were visualized using Gel-Doc UV trans-illuminator and sizing done using 100 bp DNA ladder, Invitrogen.

Data Analysis We carried out two statistical tests. First, we used the Mann–Whitney test to compare proportion of isolates that were resistant against each antibiotic between humans and rhinoceros ($n = 8$ corresponding to the number of antibiotics we used). Second, and as a continuation of the first test, we used binary logistic regression to compare the odds of antibiotic resistance in rhinoceros compared to that in humans. For the binary logistic regression, we counted the number of isolates that were resistant to each antibiotic to enable us carry out the test for each antibiotic agent. We used humans as the reference group for each of the eight tests corresponding to eight antibiotics that we tested. All statistical tests were performed in SPSS Version 17 and significance was evaluated at p value ≤ 0.05.

Results

In this study, a total of 184 and 16 fecal samples were collected from humans and black rhinoceros, respectively. *Escherichia coli* was isolated in all the 184 human samples and in 15 of the black rhinoceros samples; one isolate per fecal sample with the exception of one rhinoceros fecal sample for which we were unable to isolate *E. coli*.

Antibacterial Resistance in *Escherichia coli* Isolates from Black Rhinoceros and Humans

Escherichia coli isolates from human and black rhinoceros showed varied degree of susceptibility *against* amoxicillin/clavulanic acid (human and rhinoceros: 85.3% and 60%), cotrimoxazole (83.1% and 33.3%), gentamicin (28.3% and 80%), erythromycin (76.1% and 86.7%) and ampicillin (75.0% and 73.3%), tetracycline (64.7% and 40.0%), ceftriaxone (58.3% and 13.3%) and chloramphenicol (29.9% and 6.7%), respectively, (Table 2).

Ampicillin, tetracycline, amoxicillin/clavulanic acid and erythromycin resistance were high in *E. coli* isolated from both human and black rhinoceros. Isolates from black rhinoceros were highly susceptible to chloramphenicol. At a first pass, the observed antibiotic resistance in six out of the eight antibiotic agents tested was higher in *E. coli* strains isolated from human than in those from black rhinoceros (Fig. 1). Two of the eight antibiotic agents tested (erythromycin and gentamicin) gave an unexpected results

Table 1. Primer Sequences Used for PCR Amplification.

Antimicrobial Agent	Gene	Sequence	Annealing temp (°C)	References
Beta-lactams	*blaTEm*	**(F)** CATTTCCGTGTCGCCCTTAT **(R)** TCCATAGTTGCCTGACTCCC	55	Van et al. (2008)
Sulfonamide	*Sul1*	**(F)**TTCGGCATTCTGAATCTCAC **(R)**ATGATCTAACCCTCGGTCTC	63	Van et al. (2008)
Trimethoprim	*dfrA1*	**(F)**AAGAATGGAGTTATCGGGAATG **(R)**GGTAAAAACTGGCCTAAAATTG	58	Van et al. (2008)
Tetracycline	Tet(A)	**(F)** GGTTCACTCGAACGACGTCA **(R)** CTGTCCGACAAGTTGCATGA	57	Aarestrup et al. (2003)
	tet(B)	**(F)** CCTCAGCTTCTCAACGCGTG **(R)** GCACCTTGCTGATGACTCTT	56	Aarestrup et al. (2003)

Table 2. Antibiotic Resistance Profile of *Escherichia coli* Isolates from Black Rhinoceros in Lambwe Valley, Kenya.

Antibiotic agent	% Susceptibility in humans (n = 184)			% Susceptibility in black rhinoceros (n = 15)		
	R	*I*	*S*	*R*	*I*	*S*
Ampicillin	75.0	11.4	13.6	73.3	26.7	0.0
Gentamicin	28.3	23.4	48.3	80.0	6.7	13.3
Tetracycline	64.7	13.0	22.3	40.0	6.7	53.3
Cotrimoxazole	83.1	4.4	12.5	33.3	0.0	66.7
Chloramphenicol	29.9	24.5	45.6	6.6	6.7	86.7
Ceftriaxone	58.3	20.3	21.4	13.3	0.0	86.7
Amoxycillin/clavulanic acid	85.3	12.5	2.2	60.0	13.3	26.7
Erythromycin	76.1	19.0	4.9	86.7	13.3	0.0

R resistance, *I* intermediate, *S* sensitive.

where proportion of antibiotic resistance was higher in *E. coli* strains isolated from black rhinoceros than those from humans (Fig. 1). Despite the phenotypic differences observed in antibiotic resistant *E. coli* between human and black rhinoceros, the differences in proportion of isolates that showed resistant against each antibiotic were not statistically significant (Mann–Whitney test $U = 25$, $p = 0.462$).

However, further statistical interrogation using binary logistic regression showed that there was no significant association between antibiotic resistance patterns and species in 50% of the antibiotic agents (ampicillin, $X^2 = 0.020$, $p = 0.886$, OR = 0.917 95% CI = 0.278 to 3.019; tetracycline $X^2 = 3.384$, $p = 0.066$, OR = 0.363, 95% CI = 0.124 to 1.068; chloramphenicol $X^2 = 2.824$, $p = 0.093$, OR = 0.172, 95% CI = 0.022 to 1.340) and erythromycin $X^2 = 0.841$, $p = 0.359$, OR = 2.043, 95% CI = 0.444 to 9.404). In contrast, we found significant association between antibiotic resistance and species in the remaining 50% of the antibiotics (cotrimoxazole $X^2 = 15.473$, $p = 0.000$, OR = 0.101, 95% CI = 0.032 to 317), ceftriaxone $X^2 = 7.921$, $p = 0.005$, OR = 0.113, 95% CI = 0.025 to 0.516, amoxicillin/clavulanic acid $X^2 = 5.716$, $p = 0.017$, OR = 0.258, 95% CI = 0.085 to 0.783 and gentamycin $X^2 = 12.114$, $p = 0.001$, OR = 10.154, 95% CI = 2.753 to 37.452). Seventy-five percent of antibiotic agents which exhibited differences in resistance patterns between the two species (cotrimoxazole, ceftriaxone and amoxicillin/clavulanic acid) pointed to higher antibiotic resistance pattern in isolates from humans than in black rhinoceros, whereas gentamicin resistance was higher in isolates from black rhinoceros than those from humans.

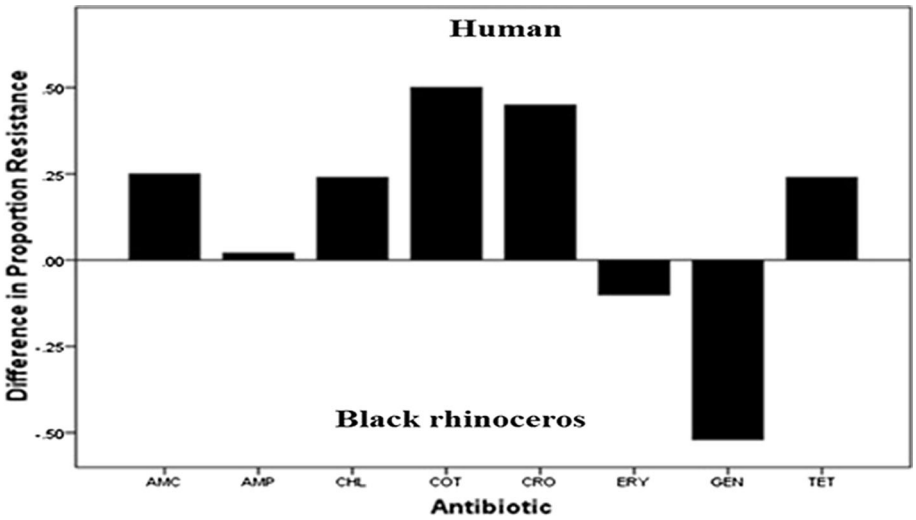


Figure 1. Differences in proportions of antibiotic resistance in *E. coli* isolates from human and black rhinoceros in Lambwe Valley. Amoxicillin/clavulanic acid (AMC), ampicillin (AMP), chloramphenicol (CHL), cotrimoxazole (COT), ceftriaxone (CRO), erythromycin (ERY), gentamycin (GEN) and tetracycline (TET). Difference in proportion of antibiotic resistance between the two species was obtained by subtracting proportions of isolates that showed resistance in black rhinoceros from those of humans. A positive value means resistance was higher in humans while negative value means resistance was higher in black rhinoceros.

Resistance to more than one class of antimicrobial agent was identified as representing as a case of multi-drug resistance (MDR). The proportion of MDR phenotype of *E. coli* isolated from black rhinoceros was 43.4%, while that from human isolates was 69% (Table 3).

Antibiotic Resistance Genes in *E. coli* Isolates from Human and Black Rhinoceros

Six isolates from humans and 4 from black rhinoceros were randomly chosen from a purposively selected pool of isolates that showed high resistance across all tested antibiotics were subjected to molecular analysis for detection of bla_{TEM}, *tetA* and *tetB*, *Sul1* and *dfrA1* genes. All the 6 selected isolates resistant to ampicillin from human (100%) and 3 of 4 (75%) from black rhinoceros gave positive amplicons for bla_{TEM} genes (Fig. 2a). For tetracycline resistance, 4 of 6 (67%) isolates from human and 3 of 4 (75%) isolates from black rhinoceros had *tetA* genes (Fig. 2b), while one isolate each from human and black rhinoceros, that is 16% of isolates from humans and 25% of isolates from black rhinoceros, were positive for *tetB* genes (Fig. 2c). For cotrimoxazole, 2 of 6 (33%) isolates from human and 1 of 4 isolates (25%) from black rhinoceros had *dfrA1* genes (Fig. 2d), while 3 of 6 isolates (50%) from human and 1 of 4 isolates (25%) from black rhinoceros expressed *sul1* genes (Fig. 2e).

Discussion

To the best of our knowledge, this is the first study to compare antibiotic resistance profiles between a wild population of the critically endangered black rhinoceros and humans. The results of this study show that there is similarity in antibiotic resistance in *E. coli* isolated from human and black rhinoceros in Lambwe Valley, Kenya. More specifically, there was no difference in antibiotic resistance between the two species in 50% of the antibiotics we tested.

The antibiotic resistance profiles for the four antibiotics where difference in resistance was not found suggest that the two species may be exposed to the same selective pressure or exchange genes that confer antibiotic resistance through commensal bacteria such as *E. coli*. A possible pathway here would be that humans are exposed through the traditional contacts of prescriptions or over-the-counter medications and consumption of animal products containing genes that confer antibiotic resistance. On the other hand, black rhinoceros may be exposed to anthropogenic sources of antibiotic resistance genes such as contaminated human wastes and effluents from sewage water (Radhouani et al. 2014). Environmental sources of antibiotic resistant genes may be of particular importance in the Lambwe Valley as is the case in much of the developing world where waste management remains a challenge.

Table 3. Multi-drug Resistance Pattern of *E. coli* Isolates from Humans and Black Rhinoceros in Lambwe Valley.

Resistant to:	Species	Resistant phenotype	Number of isolates
1 Antibiotic	Black rhinoceros		0
	Human	*Cot*	1
		Cro	1
2 Antibiotics	Black rhinoceros	*Amp, Ery*	1
		Tet, Cro	1
		Gen, Ery	2
		Amp, Gen	1
	Human	*Cot, Amc*	6
		Amp, Cot	2
		Amc, Ery	3
		Chl, Amc	1
		Cro, Amc	1
		Cot, Ery	3
		Amp, Amc	2
		Tet, Cot	1
		Tet, Ery	1
3 Antibiotics	Black rhinoceros	*Amp, Gen, Ery*	1
		Amp, Amc, Ery	1
		Gen, Amc, Ery	1
	Human	*Amp, Amc, Ery*	3
		Tet, Cro, Amc	1
		Gen, Amc, Ery	2
		Amp, Cot, Ery	3
		Tet, Cot, Cro	3
		Cot, CAmc, Ery	2
		Tet, Cot, Amc	2
4 Antibiotics	Black rhinoceros	*Amp, Gen, Amc, Ery*	1
	Human	*Amp, Cro, Amc, Ery*	7
		Tet, Cot, Amc, Ery	6
		Amp, Tet, Cot, Amc	2
		Amp, Cot, Cro, Ery	2
		Amp, Tet, Amc, Ery	3
		Amp, Cot, Cro, Amc	2
		Gen, Cot, Cro, Ery	1
		Tet, Cot, Cro, Amc	1
		Amp, Tet, Cot, Cro	1
		Amp, Cot, Amc, Ery	2
		Tet, Chl, Amc, Ery	1
		Amp, Gen, Amc, Ery	1
		Amp, Cot, Chl, Ery	1
		Amp, Chl, Cro, Ery	1

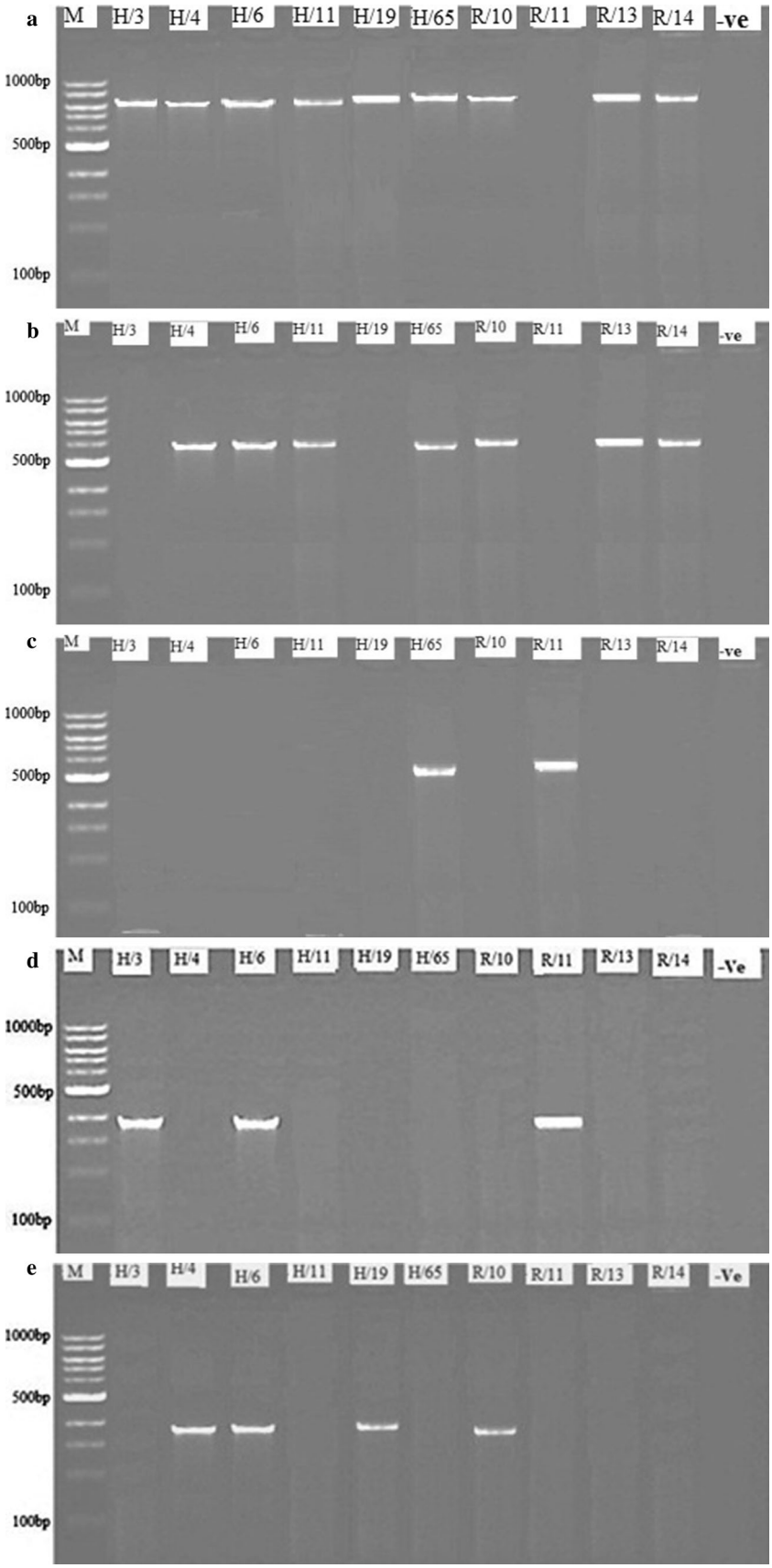
Table 3. continued

Resistant to:	Species	Resistant phenotype	Number of isolates
5 Antibiotics	Black rhinoceros	*Amp, Gen, tet, Amc, Ery*	1
	Human	*Amp, Cot, Cro, Amc, Ery*	4
		Amp, Tet, Cot, Cot, Amc	5
		Amp, Gen, Tet, Cot, Amc	3
		Amp, Tet, Cot, Amc, Ery	16
		Tet, Cot, Chl, Amc, Ery	2
		Amp, Tet, Cro, Amc, Ery	1
		Amp, Cot, Chl, Amc, Ery	1
		Amp, gen, Cot, Amc, Ery	1
		Amp,Chl, Cro, Amc, Ery	1
		Amp, Tet, Cot, Cro, Ery	1
		Tet, Cot, Chl, Cro, Amc	1
		Amp, Gen, Chl, Cro, Amc	1
		Gen, Tet, Cot, Cro, Ery	1
6 Antibiotics	Black rhinoceros	*Amp, Gen, Cot, Cro, Amc, Ery*	1
		Amp, Tet, Cot, Cro, Amc, Ery	3
	Human	*Gen, Tet, Cot, Cro, Amc, Ery*	1
		Amp, Tet, Chl, Cro, Amc, Ery	1
		Amp, Tet, Cot, Chl, Amc, Ery	4
		Amp, Tet, Cot, Cro, Amc, Ery	13
		Amp, Tet, Cot, Chl, Cro, Amc	2
		Amp, Gen, Cot, Cro, Amc, Ery	4
		Amp, Gen, Tet, Cot, Cro, Amc	2
		Amp, Cot, Chl, Cro, Amc, Ery	1
		Amp, Gen, Tet, Cot, Amc, Ery	2
		Amp, Gen, Chl, Cro, Amc, Ery	1
		Tet, Cot, Chl, Cro, Amc, Ery	3
7 Antibiotics	Black rhinoceros	*Amp, Gen, Tet, Cot, Chl, Amc, Ery*	1
	Human	*Amp, Tet, Cot, Chl, Cro, Amc, Ery*	9
		Amp, Gen, Tet, Cot, Cro, Amc, Ery	7
		Gen, Tet, Cot, Chl, Cro, Amc, Ery	2
8 Antibiotics	Black rhinoceros		0
	Human	*Amp, Gen, Tet, Cot, Chl, Cro, Amc, Ery*	21

AMC amoxicillin/clavulanic acid, *AMP* ampicillin, *CHL* chloramphenicol, *COT* cotrimoxazole, *CRO* ceftriaxone, *ERY* erythromycin, *GEN* gentamicin, *TET* tetracycline.

Any contaminants in Lambwe River that passes through the park may be a major point where black rhinoceros are exposed to genes that confer antibiotic resistance. Alternatively, antibiotic resistance in black rhinoceros may derive from environmental bacteria as a function of chronic exposure of such bacteria to environmental stressors such as heavy metals (Davies and Davies 2010).

Since the presence of similar antimicrobial resistance genes in both humans and wildlife (rhinoceros for our case) is not surprising, it must be appreciated that there exists a complex network of antimicrobial resistance transmission routes between human, livestock, wildlife and environment (Martinez 2009; Davies and Davies 2010; Allen et al. 2011). For instance, food contaminated with rodent feces and ingested by humans or animals, exchange of genes between environmental bacteria and human pathogens in aquatic systems (Cabello et al. 2013; Wellington et al. 2013), contact during treatment by veterinarians or hunting and trapping of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◀**Figure 2.** **a** A micrograph of *blaTEM* gene (857 bp), **b** a micrograph of *TetA* gene (577 bp), **c** a micrograph of *TetB*gene (634 bp), **d** a micrograph of *dfrA1* gene (391 bp) and **e** a micrograph of *sul1* gene (350 bp) in *Escherichia coli* isolates from human and black rhinoceros. M = DNA ladder, H/3, H/4, H/6, H/11 and H/65 represent human samples and R/10, R/11, R/13 and R/14 represent black rhinoceros samples; − Ve = negative control.

wild animals are important pathways in antimicrobial resistance exchanges. Moreover, social association in herbivores such as giraffe has been shown to promote the spread of resistant *E. coli* (Miller et al. 2019). Possibilities of rhinoceros acquiring resistance genes from such close species are very high. Therefore, the hypothesis that both human and rhinoceros have similar phenotypic antimicrobial patterns must be considered with a lot of caution.

The public health threat of antibiotic resistance is exacerbated by the fact that genes that confer antibiotic resistance can be transferred between species, horizontal transfer, through several mobile genetic elements including plasmids, integrons and transposons. For instance, resistance to chloramphenicol is usually associated with chloramphenicol acetyltransferases which inactivate chloramphenicol or efflux chloramphenicol via specific membrane-associated transporters that are linked to transposons, integrons and plasmids. These agents of horizontal transfer of genes that confer antibiotic resistance may account for the similarity in chloramphenicol resistance we have reported between humans and black rhinoceros. In this regard, the absence of difference in chloramphenicol resistance between the two species could be as a result of either adaptive or acquired resistance mechanisms that contribute to the tolerance of antibiotics (Fernandez et al. 2011; Skiada et al. 2011; de la Fuente-Núñez et al. 2013; Chong et al. 2018; Prüss-Ustün et al. 2014; Woerther et al. 2013).

We also found notable differences in antibiotic resistance between the two species. Resistance to contrimoxazole, ceftriaxone and amoxicillin/clavulanic acid was higher in humans than in rhinoceros. Nonetheless, an interesting result from the current study was the unexpectedly high level of resistance to gentamicin in *E. coli* isolates from black rhinoceros. The observed gentamicin resistance may be attributed to environmental factors such as the production of this antibiotic by *Micromonospora purpureochromogenes,* which is widely present in the environment (in both water and soil) as an important saprotrophic bacterium (Kumar et al. 2008). The higher level of resistance to gentamycin by *E. coli* isolates from black rhinoceros than in humans is inconsistent with the commonly held view that antibiotic resistance is transmitted from anthropogenic sources to the wild. Instead, it supports research that have demonstrated emergence and spread of genes that confer antibiotic resistance from the environment to humans (Davies and Davies 2010).

In regard to genes that confer antibiotic resistance, *E. coli* isolates from humans and black rhinoceros contained bla_{TEM}, *tetA*, *tetB*, *dfrA1* and *sul1* genes. These results also point to the possibility that both species may be under similar selective pressure or experience some degree of gene transfer that confers antibiotic resistance. We caution however that since we purposively selected a small sample of genes to characterize, the presence of antibiotic resistance genes in *E. coli* isolates from human and black rhinoceros should not be taken to correspond to the burden of antibiotic resistance genes in the two populations. Furthermore, we note that although we focused our assays for genes known to confer antibiotic resistance on isolates that were resistant, it has been shown that even isolates that are susceptible to antibiotics also contain respective antibiotic resistance genes (Turchi et al. 2019). However, we wish to note that the origin and mechanisms of resistance were postulated but not determined as these would have required phylogenetic and functional analysis of the bacterial genome, which was beyond the scope of the present study.

The antibiotic resistance genes that we targeted, bla_{TEM}, *tetA*, *tetB*, *dfrA1* and *sul1*, have previously been isolated from birds, mammals, reptiles, invertebrates, fish, humans, among other animals (reviewed in Radhouani et al. 2014; Vittecoq et al. 2016). A few examples include wild rabbits in Northern Portugal (Silva et al. 2010), dogs and wild birds (Jamborova et al. 2018), black-headed sea gulls in Czech Republic (Dolejska et al. 2007), giraffe (Miller et al. 2019) and humans (Jamborova et al. 2018; Kipkorir et al. 2016). Collectively, the wide distribution of these genes in companion animals, livestock, wildlife and in humans not only points to shared selective pressures or potent conduits that facilitate their transfer across different hosts but also to the unprecedented threat antibiotic resistance poses to public health and wildlife conservation.

Conclusion

These findings contribute to the increasingly growing corpus of literature on antibiotic resistance at the human–

wildlife interface. The presence of genes that confer resistance to commonly used antibiotics in both humans and black rhinoceros is not surprising but could be a pointer to a possibility of cross-transfer of antibacterial resistance genes between the two species. Alternatively, the two species may be under similar selective pressures. In sum, findings from the present study call for a multi-sectorial coordinated action plan on surveillance of antibiotic resistance. Further, it would be informative to establish the phylogenetic groups and patterns of transfer of *E. coli* isolated from humans and black rhinoceros so as to explicate the similarities and differences of antibiotic resistance observed in the present study. This knowledge can be useful for development of treatment strategies to tackle bacterial diseases that may imperil both public health and wildlife species such black rhinoceros that are under threat of extinction.

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