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ORIGINAL ARTICLE

Assessment of three indigenous South African herbivores as potential reservoirs and vectors of antibiotic-resistant *Escherichia coli*

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Abstract Due to limited data available on the presence of antibiotic-resistant (ABR) bacteria in faeces of wild herbivores in South Africa, this study analysed resistance patterns for *Escherichia coli* isolates from wildebeest, zebra and giraffe in addition to pet and farm pig faeces. Total and faecal coliforms and *E. coli* were quantified in faecal matter using a most probable number (MPN) guideline procedure. Antibiotic resistance profiles against 12 selected antibiotics representing seven classes were determined for 30 randomly selected *E. coli* isolates from each animal using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) disk diffusion procedure. While $\log_{10}$ MPN values per gram of animal faeces for total/faecal coliforms ranged from 4.51/4.11 to 5.70/5.50, the *E. coli* MPN values were in a range of 3.43–5.14. The proportion of ABR *E. coli* isolates ranged from 43% (giraffe) to 93% (zebra). About 47% of *E. coli* isolates from zebra faeces were categorized as multidrug-resistant (MDR), while for wildebeest and giraffe, no MDR isolates were detected. In comparison, 10% of *E. coli* isolates from pet pig and about 7% from farm pig faeces were categorized as MDR. Although most MDR isolates were resistant to at least one β-lactam antibiotic, only one MDR isolate from farm pig faeces was resistant to both norfloxacin and ciprofloxacin, the two fluoroquinolones tested. However, no resistance was detected to the tested carbapenems and tigecycline. The results of this study indicate that indigenous South African herbivores may serve as potential reservoirs and vectors for the dissemination of ABR *E. coli* strains.

Keywords *Escherichia coli* · South Africa · Faeces · Indigenous herbivores · Antibiotic resistance · Multidrug resistance

Introduction

The extensive use of antibiotic compounds in human and veterinary medicine has led to the presence of antibiotic residues in food and the environment (Welch 1957; Kümmerer 2003; Kemper 2008; Martinez 2009). Consequently, recent research has focused on the transfer and detection of antibiotic-resistant microorganisms and their genes in the environment (Sørum and L'Abée-Lund 2002; Garcia-Alvarez et al. 2012). Only recently, the World Health Organization (WHO), the European Food and Safety Authority (EFSA) and even the President of the USA highlighted antibiotic resistance as a serious global health problem (White House 2014; WHO 2014; EFSA 2017). As zoonotic bacteria can acquire resistance to antibiotics typically utilized in human and veterinary medicine, farm animals can serve as a potential reservoir for antibiotic-resistant (ABR) bacterial strains as well as potential vectors for transferring such resistant bacteria or their genes into the environment (Sørum and L'Abée-Lund 2002; Silbergeld et al. 2008; EFSA 2017). Zoonotic bacteria presenting resistance to antibiotics critical in human medicine such as fluoroquinolones and third and fourth generation cephalosporins are of particular concern, as they may compromise the effective treatment of infections in humans (Hammerum and Heuer 2009; WHO 2016). Humans can acquire infections with such antibiotic-resistant zoonotic bacteria either via

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contact with animals or through ingestion of contaminated food products of animal and non-animal origin.

Escherichia coli is an intestinal bacterium commonly associated with the digestive system of animals and therefore considered a useful hygiene indicator (Leclerc et al. 2001). However, pathogenic strains of *E. coli* can be a serious threat to public health as was observed in 2011 and 2016, when Shiga-toxin-producing strains of *E. coli* caused outbreaks in European countries, ultimately attributed to the consumption of sprouts made from contaminated fenugreek seeds (EFSA 2011) and soft cheese (Peron et al. 2016). The pathogenic *E. coli* strain causing the 2011 outbreak exhibited multidrug resistance against ampicillin, ceftriaxone, streptomycin and tetracycline (King et al. 2012), further highlighting the problem of multidrug-resistant (MDR) *E. coli*.

Antibiotic usage in the diets of food animals as prophylactic or therapeutic means can promote the horizontal transfer of antibiotic resistance genes (Silbergeld et al. 2008; Wellington et al. 2013), which might even take place in the absence of selective pressure imposed by the use of antibiotics (Allen et al. 2010). Human-livestock contact taking place in rural areas in developing countries might promote exposure to and transfer of such zoonotic bacteria due to the close contact between the population and livestock (Rwego et al. 2008; Klous et al. 2016). Additionally, studies in South Africa by Schellack et al. (2011) indicated that social issues including widespread poverty and lack of access to safe potable water and proper sanitation might stimulate the dissemination of MDR bacterial strains. However, ABR bacteria are even found in the faeces of wild animals, ranging from primates (Rolland et al. 1985) and rodents (Guenther et al. 2010) to wild boars (Literak et al. 2010) and various wild birds (Costa et al. 2008), with even extended spectrum β-lactamase (ESBL) containing *E. coli* being detected (Literak et al. 2010). It was assumed that the presence of such ABR bacteria is most likely due to anthropogenic activities or contact with anthropogenic waste material; for example, seagulls that scavenge food wastes (Cole et al. 2005; Radhouani et al. 2009) or wild baboons feeding on human refuse (Rolland et al. 1985). The ribotyping patterns of the majority of *E. coli* isolates from seagulls in New Hampshire (USA) had a ≥90% similarity to those of *E. coli* strains isolated from proximate wastewater treatment plants and landfill sites (Nelson et al. 2008), suggesting a potential for transfer of faecal bacteria to recreational areas frequented by humans. Moreover, considering the ability of long distance migration, birds can act as efficient transporters for the dissemination of antibiotic-resistant bacteria and mirror the spectrum of such bacteria found in humans and human waste materials (Radhouani et al. 2009).

Antibiotic resistance profiles of *E. coli* isolates from various African wild animals—ranging from buffalo, eland and primates to the smaller mongoose and warthog—have been reported (Rolland et al. 1985; Skurnik et al. 2006; Pesapane et al. 2013; Jobbins and Alexander 2015; Katakweba et al. 2015). However, to the best of our knowledge, no information on the resistance profiles of *E. coli* from the three herbivores zebra, giraffe and wildebeest is available for KwaZulu-Natal, South Africa. To estimate the extent of antibiotic resistance among bacteria associated with these herbivores, which might serve as potential reservoirs and vectors, surveillance is necessary. Therefore, this study reports initial screening data pertaining to antibiotic resistance profiles of *E. coli* isolates from the three selected indigenous herbivores zebra, giraffe and wildebeest, in comparison to farm and pet pig.

Materials and methods

Sample collection

Faecal samples (pooled from at least three faecal samples per animal) of giraffe (*Giraffa camelopardalis*), zebra (*Equus burchellii*) and wildebeest (*Connochaetes taurinus*) were obtained from the Bisley Valley Nature Reserve (S29°66′18.99″ E30°39′10.95″), while pig (*Sus scrofa domesticus*) faeces were collected from a commercial pig farm in KwaZulu-Natal and a private household (pig kept as a pet) situated in Pietermaritzburg (KwaZulu-Natal, South Africa). All samples were collected between February and May 2015 using sterile, labelled plastic bags and immediately transferred on ice to the laboratory with sample analysis taking place within 12 h.

Quantification of total and faecal coliforms and *E. coli* from faecal samples

Enumeration of total and faecal coliforms as well as *E. coli* was carried out in a single analysis for each of the five faecal samples according to the most probable number (MPN) guideline procedure MFHPB-19 (Health Canada 2002). Confirmation of presumptive *E. coli* isolates was done via biochemical confirmation (GIMViC) and by PCR as described previously (Gemmell and Schmidt, 2012). For the PCR-based detection of the glutamate decarboxylase A (*gad*A) gene, cells were harvested by centrifugation (13,500×*g*, 5 min) from 1 ml of overnight cultures of presumptive *E. coli* isolates (nutrient broth, 35 °C, 120 rpm), re-suspended in 100 μl sterile water followed by DNA extraction using a simple freeze and thaw technique (Gemmell and Schmidt 2012). The amplification reactions, using primers reported by Kim et al. (2006), were done in 25 μl volumes, containing 1.5 μl template DNA, 0.5 μl of each primer (10 μM, Inqaba), 12.5 μl 2× DreamTaq Green PCR Master Mix (Thermo Scientific) and nuclease-free water (KAPA), with the following cycling conditions (Labnet MultiGene II thermocycler): initial denaturation at 94 °C for 2 min followed

by 25 cycles at 94 °C for 30 s, annealing at 55 °C for 30 s, 72 °C for 1 min and a final extension cycle at 72 °C for 7 min. PCR products were analysed by electrophoresis on 1% (*w*/*v*) agarose gel with addition of SYBR Safe stain (Life technologies) and visualized under UV light using a Gbox Chemi XRQ system (Syngene) and GeneSnap software. A ready-to-use 100 bp DNA ladder (KAPA) was used as size marker, and *E. coli* ATCC 8739 served as positive PCR control with *Salmonella* Typhimurium ATCC 14028 and sterile Milli-Q water employed as negative controls.

Antibiotic susceptibility disk diffusion assay

The disk diffusion method (version 5, 2015) from the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2015) was used to assess the antibiotic resistance patterns of *E. coli* isolates. The following 12 antibiotics were tested employing ready-to-use 6-mm disks (Oxoid): ampicillin (AMP 10 μg), amoxicillin-clavulanic acid (AMC 20/10 μg), cefotaxime (CTX 5 μg), ceftazidime (CAZ 10 μg), ertapenem (ERT 10 μg), meropenem (MEM 10 μg), aztreonam (ATM 30 μg), ciprofloxacin (CIP 5 μg), norfloxacin (NOR 10 μg), gentamicin (GEN 10 μg), tobramycin (TOB 10 μg) and tigecycline (TIG 15 μg). Antibiotics utilized in this study were categorized into classes based on therapeutic relevance, as suggested by Magiorakos et al. (2012). For the disk diffusion assay, suspensions from each *E. coli* isolate were prepared from nutrient broth cultures after incubation at 35 °C (120 rpm, 14 h) and adjusted to approximately 2×10^8 cells per ml (corresponding to approximately 0.5 McFarland standard suggested by EUCAST) using a Helber-type bacterial counting chamber (Marienfeld, Germany). One hundred microlitres of cell suspension was spread plated onto Mueller-Hinton agar (Oxoid) plates containing 25 ml of agar per sterile 90 mm Petri dish. Four different antibiotic test disks (adjusted for approximately 1 h to ambient temperature) were firmly placed equidistantly onto the agar surface. Plates were sealed using Parafilm and incubated inverted in stacks of two at 35 °C for 20 h as specified (EUCAST 2015). Inhibition zones were measured to the nearest millimetre using digital Vernier callipers (Marshall Tools, India) and analysed using the EUCAST antibiotic breakpoint tables (EUCAST 2016). All isolates were analysed in duplicate against all 12 antibiotics.

Chemicals

Unless otherwise stated, all chemicals used were of the highest purity commercially available.

Results

In this study, both total and faecal coliforms as well as *E. coli* were quantified in faeces collected from zebra, wildebeest, giraffe and farm and pet pig. The MPN values of total coliforms for wild herbivores were in the range from 4.51 to 5.36 $\log_{10}$ MPN/g, while values for pig faeces were slightly higher at 5.50 and 5.70 $\log_{10}$ MPN/g (Table 1). Faecal coliform levels were lower than the corresponding total coliform values, ranging from 4.11 (wildebeest) to 5.50 $\log_{10}$ MPN/g (pet pig) (Table 1). The $\log_{10}$ MPN/g values for *E. coli* in zebra, wildebeest and giraffe faeces were 4.69, 3.43 and 4.89 $\log_{10}$ MPN/g, respectively, while the values for pet and farm pig faeces were slightly higher at 5.11 and 5.14 $\log_{10}$ MPN/g (Table 1).

From each of the five tested animals, 30 randomly selected confirmed faecal *E. coli* isolates were further analysed for their antibiotic resistance patterns. In total, 106 out of 150 selected *E. coli* isolates from all animal faeces were resistant to at least one of the 12 antibiotics tested (Tables S1 and 2). The highest proportion of *E. coli* isolates showing resistance to at least one antibiotic was found in zebra faeces (93%) followed by isolates from pet pig and wildebeest faecal samples, with 90 and 80% resistance. In contrast, isolates from farm pig and giraffe showed the lowest proportion of resistances with 47 and 43%, respectively (Table 2). More than half (56.6%) of all 106 *E. coli* isolates from wild herbivore and pig faeces presenting antibiotic resistance possessed only one resistance, followed by about 23% (24/106 isolates) with a combination of two antibiotic resistances. The remaining 22 *E. coli* isolates showed resistances ranging from three (19 isolates) up to even five (one isolate) antibiotic compounds. With few exceptions (four isolates), all antibiotic-resistant (ABR) *E. coli* isolates possessed resistance against at least one of the following tested β-lactam antibiotics: ampicillin, amoxicillin-clavulanic acid, ceftazidime, cefotaxime or aztreonam (Table S1). Of all antibiotics tested, amoxicillin-clavulanic acid was the least effective as a large proportion (94 isolates) of the ABR *E. coli* isolates obtained from all five animals showed resistance against this compound (Fig. 1). However, no resistances were observed for the two tested carbapenems ertapenem and meropenem, the cephalosporin cefotaxime and the glycylcycline tigecycline (Table S1). Interestingly, only six out of all 106 ABR *E. coli* isolates were resistant to ampicillin, 50% of which originated from farm pig faeces (Table S1). The proportion of ceftazidime-resistant *E. coli* isolates from zebra faeces was clearly higher than that observed in all other animals (Fig. 1). Among the non-β-lactam antibiotics tested, the least effective was the aminoglycoside compound tobramycin and, to a lesser degree, gentamicin (Fig. 1). Noticeably, one out of all 150 *E. coli* isolates analysed—from farm pig faeces—presented resistance to both ciprofloxacin and norfloxacin, the two fluoroquinolones tested.

Table 1 Quantification of total and faecal coliforms and *Escherichia coli* (with 95% confidence intervals) in pooled faecal samples obtained from wild herbivores and pet and farm pigs

Source of faeces	Total coliforms (log_{10} MPN/g)	95% CI (lower/upper limit)	Faecal coliforms (log_{10} MPN/g)	95% CI (lower/upper limit)	*E. coli* (log_{10} MPN/g)	95% CI (lower/upper limit)
Zebra	5.11	4.66 / 5.57	4.89	4.40 / 5.38	4.69	4.19 / 5.19
Wildebeest	4.51	4.04 / 4.99	4.11	3.65 / 4.56	3.43	3.06 / 3.80
Giraffe	5.36	4.89 / 5.83	5.11	4.66 / 5.57	4.89	4.40 / 5.38
Farm pig	5.70	5.19 / 6.20	5.34	4.95 / 5.72	5.14	4.74 / 5.54
Pet pig	5.50	5.03 / 5.97	5.50	5.03 / 5.97	5.11	4.66 / 5.57

The prevalence of multidrug-resistant (MDR) *E. coli* isolates—those showing resistance to antibiotics from at least three different classes—was considerably lower with 19 MDR isolates detected among the 150 *E. coli* isolates analysed (Table 2). Approximately 73% (14/19) of all these MDR isolates originated from zebra faeces (hence 47% of the 30 zebra *E. coli* isolates), while the analysis of giraffe and wildebeest faeces resulted in no *E. coli* isolates with multiple drug resistance. The analysis of pet pig faeces established 10% (3 out of 30) MDR isolates. Interestingly, the proportion of MDR *E. coli* isolates obtained from farmed pig was also in the lower range at about 7% (2 out of 30). The phenotypic resistance profiles for all MDR *E. coli* isolates are summarized in Table 3. Nearly all MDR *E. coli* isolates (18 out of 19) featured resistance against the antibiotic amoxicillin-clavulanic acid, and 11 isolates (8 isolates from zebra and 3 from pet pig) showed the same resistance profile of amoxicillin-clavulanic acid, ceftazidime and tobramycin (AMC-CAZ-TOB). One MDR *E. coli* isolate from farm pig faeces was resistant to five different antibiotics: amoxicillin-clavulanic acid, ampicillin, aztreonam, norfloxacin and ciprofloxacin (AMC-AMP-ATM-NOR-CIP). Moreover, this was the only *E. coli* isolate displaying resistance to both fluoroquinolones tested.

However, high levels of intermediate resistance were observed for the aminoglycoside antibiotics gentamicin (136 isolates) and tobramycin (116 isolates), as well as for the β-lactam antibiotics ceftazidime (101 isolates) and aztreonam (98 isolates) (Table S1). In contrast to this, only one *E. coli* isolate—from wildebeest faeces—presented an intermediate resistance phenotype for tigecycline. In addition, less than 10 isolates showed an intermediate phenotype for the carbapenem antibiotic meropenem and the fluoroquinolones norfloxacin and ciprofloxacin. As the EUCAST breakpoint tables (2016) do not provide an intermediate resistance category for ampicillin and amoxicillin-clavulanic acid, intermediate resistance phenotypes were not established for these two antibiotics.

Discussion

As expected on microbiological grounds, the MPN values established for faeces from all five animals were higher for total and faecal coliforms than for *E. coli*. The level of *E. coli* in pet and farm pig faeces in the current study was similar to counts reported for *E. coli* in fresh pig slurry from a Bulgarian pig farm (Petkov et al. 2006) and somewhat higher than the numbers of *E. coli* established for faeces of zebra, giraffe and wildebeest (Table 1). A similar scenario was observed in France by Smati et al. (2015) when comparing *E. coli* counts in faeces of wild and domesticated animals, which was attributed to the variation in diet. Katakweba et al. (2015) quantified in Tanzania *E. coli* in zebra faeces as 4.25 log_{10} CFU/g and in wildebeest faeces as 4.60 log_{10} CFU/g, which

Table 2 Prevalence of ABR and MDR *Escherichia coli* strains isolated from faeces of wild herbivores and pet and farm pigs

Animal faeces	Number[a]	Antibiotic resistant (ABR)[b]	Multidrug resistant (MDR)[c]
Zebra	30	93% (28/30)	47% (14/30)
Wildebeest	30	80% (24/30)	0% (0/30)
Giraffe	30	43% (13/30)	0% (0/30)
Pet pig	30	90% (27/30)	10% (3/30)
Farm pig	30	47% (14/30)	7% (2/30)
Total	150	71% (106/150)	13% (19/150)

[a] Number of *E. coli* isolates analysed

[b] % of *E. coli* isolates resistant to one or more antibiotics

[c] % of *E. coli* isolates resistant to three or more antibiotics representing at least three different antibiotic classes

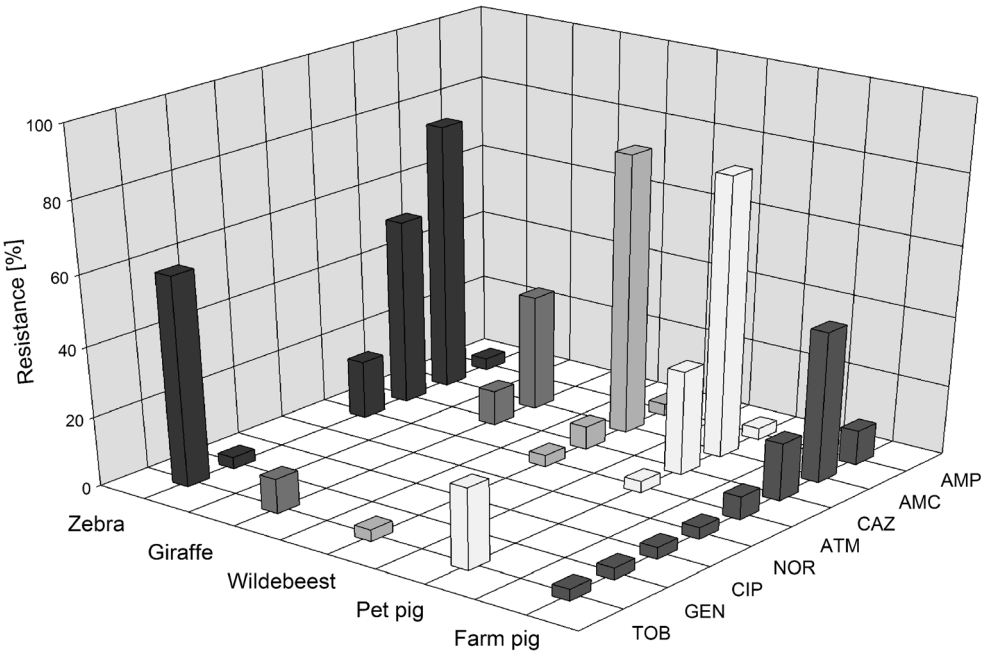

Fig. 1 Incidence of resistances observed against selected antibiotics for *Escherichia coli* isolates from zebra, giraffe, wildebeest and farm and pet pig faeces. Antibiotics for which no resistance was detected (cefotaxime, ertapenem, meropenem and tigecycline) are not shown. *AMP* ampicillin, *AMC* amoxicillin-clavulanic acid, *CAZ* ceftazidime, *ATM* aztreonam, *NOR* norfloxacin, *CIP* ciprofloxacin, *GEN* gentamicin, *TOB* tobramycin

is similar to the values obtained for the same animals in the current study.

The overall degree of resistance to one or more of the 12 tested antibiotics observed in the current study was 71%. It ranged from 43% for faecal *E. coli* isolates from giraffe to 93% for isolates from zebra. Among these faecal *E. coli* isolates from the five animals, only a small proportion exhibited ampicillin resistance (6/150). Similarly, less than 10% ampicillin-resistant *E. coli* were reported for cattle in Serbia (Knezevic and Petrovic 2008), while much higher percentage values were reported for ampicillin-resistant faecal *E. coli* isolates from wildebeest (58.8%) and zebra (47.6%) in Tanzania (Katakweba et al. 2015).

Table 3 Antibiotic resistance profiles detected for all MDR *Escherichia coli* isolates from faeces of wild herbivores and pet and farm pigs

Host species (number of MDR isolates)	Phenotypic multidrug resistance profile
Zebra (8); Pet pig (3)	AMC-CAZ-TOB
Zebra (2)	AMC-ATM-TOB
Zebra (1)	AMC-ATM-CAZ
Zebra (1)	ATM-CAZ-TOB
Zebra (1)	AMC-CAZ-GEN
Zebra (1)	AMC-ATM-CAZ-TOB
Farm pig (1)	AMC-AMP-CAZ-GEN
Farm pig (1)	AMC-AMP-ATM-NOR-CIP

AMP ampicillin, *AMC* amoxicillin-clavulanic acid, *CAZ* ceftazidime, *ATM* aztreonam, *NOR* norfloxacin, *CIP* ciprofloxacin, *GEN* gentamicin, *TOB* tobramycin

In contrast to ampicillin resistance, the degree of amoxicillin-clavulanic acid resistance among *E. coli* isolates from zebra and wildebeest in the current study was at >70% much higher than for *E. coli* isolates from wildebeest (14.7%) and zebra (11.9%) in Tanzania (Katakweba et al. 2015). Similarly, amoxicillin-clavulanic acid resistance for *E. coli* isolates from pet and farm pig in the current study amounted to 80 and 43%, respectively, while for pets and farm pigs from Spain, only 6% of isolates were resistant to the same antibiotic (Sáenz et al. 2001). Resistance levels for a given antibiotic exceeding 70% are considered as extremely high for those antibiotics considered important in human and veterinary medicine (EFSA, 2017).

Antibiotic-resistant (ABR) *E. coli* isolates were identified for the cephalosporin ceftazidime, ranging from about 7% (wildebeest) to 53% (zebra) of isolates. Similar to pets and farm pigs analysed in Spain (Sáenz et al. 2001), no resistance was detected in the current study for the cephalosporin cefotaxime, although such resistance was reported for *E. coli* isolates from herbivores in Tanzania (Katakweba et al. 2015). About 21% of all *E. coli* isolates analysed in the current study displayed intermediate resistance to cefotaxime and about 67% of the isolates for ceftazidime. This is concerning as third generation cephalosporins are considered as critically important antibiotics by the WHO (2016), and the EUCAST expert rules suggest categorizing certain intermediate resistances as resistant to avoid potential treatment failure (Leclercq et al., 2013).

For the monobactam aztreonam, resistance levels observed for *E. coli* isolates from faeces ranged from 0% for giraffe to about 17% for zebra. Again, intermediate phenotypes were detected for about 65% of *E. coli* isolates for aztreonam.

Carbapenems are intended for use in human medicine, while their application in veterinary medicine should be avoided (Poirel et al. 2014; WHO 2016). The absence of resistance against the carbapenems meropenem and ertapenem among all 150 tested *E. coli* isolates is therefore reassuring. However, several *E. coli* isolates with intermediate resistance against these two carbapenems were detected in our study. Costa et al. (2008) detected no resistance to another carbapenem, imipenem, when analysing 112 *E. coli* isolates from a large range of wild animals from nature reserves in Portugal, thereby matching our data for the three herbivores analysed in KwaZulu-Natal.

Only small numbers of ABR phenotypes were detected for the fluoroquinolones norfloxacin and ciprofloxacin. The only incidence of resistance to both ciprofloxacin and norfloxacin was detected for one *E. coli* isolate from farm pig faeces, with two additional isolates from the same animal showing intermediate resistance for ciprofloxacin and ciprofloxacin plus norfloxacin, respectively. This is in line with Sáenz et al. (2001), who identified 3% of *E. coli* isolates from farm pigs as ciprofloxacin resistant, which may be due to the use of fluoroquinolones for treating food animals (Collignon 2005). Surprisingly, *E. coli* isolates showing an intermediate phenotype for fluoroquinolones were mostly present in zebra faeces (Table S1).

Regarding aminoglycosides, the *E. coli* isolates from the five animals tested showed resistance levels ranging from 3% (farm pig, wildebeest) to 60% (zebra) for tobramycin, while not exceeding 3% for gentamicin (Fig. 1). In contrast, gentamicin resistance levels for *E. coli* from zebra and wildebeest in Tanzania were higher at 23.8 and 20.6%, while this amounted to only 3.2% in buffalo (Katakweba et al. 2015). Similar to our results for farm pig, Knezevic and Petrovic (2008) found that only 1% of *E. coli* isolated from swine presented resistance to gentamicin and tobramycin. Remarkably high levels (>75%) of intermediate phenotypes were detected for both aminoglycosides. As both gentamicin and tobramycin are approved for use in veterinary medicine in South Africa (Eagar et al., 2012), the somewhat higher incidence of tobramycin resistance in pet pig isolates in our study might be due to veterinary treatment.

While no tigecycline resistance was detected among the 150 *E. coli* isolates analysed in this study, one isolate from wildebeest faeces presented an intermediate resistance phenotype. Tetracycline resistance was reported in Africa for *E. coli* isolates from impala (Mariano et al. 2009), wildebeest, zebra and buffalo (Katakweba et al. 2015). However, tigecycline is a newer-generation tetracycline-type antibiotic not usually considered for use in veterinary medicine (Papich 2012) and typically able to overcome tetracycline resistance mechanisms (Fluit et al. 2005). Although mutations in tetracycline resistance genes can confer increased minimum inhibitory concentration values for tigecycline in *E. coli* (Linkevicius et al. 2016), no tigecycline resistance was detected among more than 360 clinical isolates of *E. coli* from South Africa, highlighting its efficiency (Kanj et al. 2014).

About 43% of antibiotic-resistant (ABR) faecal *E. coli* isolates possessed resistance against more than one antibiotic, and 19 isolates were multidrug-resistant (MDR). The highest proportion of multidrug resistance was observed for zebra faecal *E. coli* isolates at 47%, while no MDR *E. coli* isolates were detected in faeces from wildebeest and giraffe. Other studies in Africa also showed a large variability in the degree of multidrug resistance among *E. coli* isolates from different wild animals. Pesapane et al. (2013) identified 40% of faecal *E. coli* isolates from banded mongoose in the Chobe National Park (Botswana) as MDR, attributing this to contact between mongoose and guest and staff accommodations. Similar to our results, Jobbins and Alexander (2015) detected no MDR *E. coli* in giraffe faeces in Botswana. However, the same study detected 100% MDR *E. coli* when analysing spotted hyena faeces, suggesting that the variability of MDR prevalence between different animals was due to dietary factors, water-proximity and association with urban areas. Similarly, the presence of *E. coli* with extended spectrum β-lactamases (ESBL) in wild boars in Europe was attributed to their omnivorous diet and access to animal and human waste (Literak et al. 2010). Kozak et al. (2009) showed in Canada for ABR *E. coli* and Nhung et al. (2015) demonstrated in Vietnam for MDR *E. coli* phenotypes that these were manifold higher in wild mammals trapped on farms than among mammals trapped in natural areas such as forests, highlighting that antibiotic usage on farms and contact to humans contributes to antibiotic resistance.

In the present study, the incidence of faecal *E. coli* isolates resistant to at least one antibiotic was much lower in farm pig (47%) and giraffe (43%) than in pet pig (90%), wildebeest (80%) and zebra (93%). A possible reason for the higher percentage of ABR *E. coli* in pet pig faeces might be due to close contact with humans and regular veterinary care as it is well established that the treatment of pet animals can elevate antibiotic resistance levels in bacteria (Guardabassi et al. 2004).

Generally, elevated levels of ABR *E. coli* in faeces of the three herbivores analysed in the present study might be due to residents of Pietermaritzburg visiting the Bisley Valley Nature Reserve for recreational purposes. The presence of ABR *E. coli* in zebra faeces was linked in an earlier study to frequent contact between animals and staff and visitor lodging areas in Tanzania (Katakweba et al. 2015), while the transfer of antibiotic-resistant bacteria to animals has been associated with staff and their clothing (Bosman et al. 2014; Poirel et al. 2014).

Zebra and wildebeest preferentially graze close to the soil—with possible additional exposure to human waste present—while giraffe preferentially browse leaves from trees. This in addition to geophagy, which is established for herbivores and might lead to the uptake of antibiotics produced by

microorganisms present in the soil consumed (Mahaney et al. 1999), might explain to some degree why the level of faecal ABR *E. coli* was clearly higher in the grazers zebra and wildebeest than in the browser giraffe.

Additional factors potentially contributing to the presence of ABR *E. coli* in faeces from wild herbivores in the current study are (i) horses from a stable adjacent to the Bisley Valley Nature Reserve likely receiving veterinary attention that can enter the park, (ii) other animal vectors such as small mammals or migratory birds entering the park, (iii) herbicides such as glyphosate used in the proximity of the nature reserve or within it as these can cause elevated antibiotic resistance levels in *E. coli* after exposure (Kurenbach et al. 2015) and (iv) water bodies within the nature reserve with ABR *E. coli*, resistance genes or even antibiotic residues present.

The data obtained in this study show that wild herbivores from KwaZulu-Natal might serve as reservoirs and vectors for ABR and even MDR *E. coli* strains. However, further research is required in order to identify the factors involved in establishing the presence of antibiotic-resistant bacteria in the herbivores targeted in this study.

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