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First Report on a Randomized Investigation of Antimicrobial Resistance in Fecal Indicator Bacteria from Livestock, Poultry, and Humans in Tanzania

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This study provides an estimate of antimicrobial resistance in intestinal indicator bacteria from humans ($n=97$) and food animals ($n=388$) in Tanzania. More than 70% of all fecal samples contained tetracycline (TE), sulfamethoxazole (STX), and ampicillin (AMP)-resistant coliforms, while cefotaxime (CTX)-resistant coliforms were observed in 40% of all samples. The average Log_{10} colony forming units/g of CTX-resistant coliforms in samples from humans were 2.20. Of 390 *Escherichia coli* tested, 66.4% were resistant to TE, 54.9% to STX, 54.9% to streptomycin, and 36.4% to CTX. Isolates were commonly (65.1%) multiresistant. All CTX-resistant isolates contained $bla_{\text{CTX-M}}$ gene type. AMP- and vancomycin-resistant enterococci were rare, and the average concentrations in positive samples were low (log_{10} 0.9 and 0.4, respectively). A low-to-moderate resistance (2.1–15%) was detected in 240 enterococci isolates to the drugs tested, except for rifampicin resistance (75.2% of isolates). The average number of *sulII* gene copies varied between Log_{10} 5.37 and 5.68 with no significant difference between sample source, while cattle had significantly higher number of *tetW* genes than humans. These findings, based on randomly obtained samples, will be instrumental in designing antimicrobial resistance (AMR) intervention strategies for Tanzania.

Keywords: fecal indicator bacteria, antimicrobial resistance, $bla_{ctx\text{-}M}$, Tanzania

Introduction

Antimicrobial resistance (AMR) is a global threat to treatment of bacterial diseases causing dramatically increased healthcare costs due to hospitalization and expensive drug choices.[1] In sub-Saharan Africa, lack of enforcement on the control of the sale and use of antibiotics has led to widespread misuse of antimicrobials, both in human and veterinary medicine. Thus, developing countries are increasingly a source of new clones of resistant bacteria.[2,3]

Information on levels of resistance in bacteria from humans and food animal reservoirs forms the fundament for risk management of AR. However, there is generally a shortage of scientific data to demonstrate trends of AR in animal or human populations in developing countries, and there is an urgent need for systematic surveillance to compensate for this lack of information.[4]

Escherichia coli and *Enterococcus* spp. are commonly used as indicator bacteria to gauge the extent of AR in a population.[5,6] The potential of these organisms as indicators of AR has been widely demonstrated in surveillance programs implemented in developed countries.[7,8] Apart from overall resistance measured in indicator bacteria, certain important resistance phenotypes should be monitored closely, for example, *E. coli* producing extended spectrum β-lactamases (ESBLs). The often multidrug-resistant ESBL producers have emerged globally in the recent decade, giving rise to substantial treatment challenges.[9] The main type of ESBL genes in both human and animal *E. coli* belongs to the cefotaxime (CTX)-M family.[10] In Tanzania the presence of ESBL has been studied in convenience samples of bacteria from human patients,[11–13] but in animals, the prevalence is currently unknown.

Tanzania has a large livestock population, and most production systems are extensive, characterized by a low capital input and a low production level. Misuse of antibiotics is widespread mainly due to lack of knowledge on health risks and a poorly controlled prescription system.[14] So far, no systematic study of resistance level in random samples from humans and food animals in Tanzania has

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been conducted to establish baseline resistance levels in these populations. Published studies have focused on ESBL producing Enterobacteriaceae in humans in the community and hospitals[15] and in children below 2 years of age.[16] Such studies have not tried to relay the occurrence of resistant bacteria in humans to the occurrence in food animals. In other part of Africa, studies on ESBL bacteria have been carried out in humans, including studies on the prevalence in adults, children, and neonates in Ethiopia,[17] children in Guinea-Bissau,[18] children in Bangui,[19] and children in Gabon.[20]

Consequently, it is not possible from current studies to estimate whether resistance levels are indeed out of control as could be inferred from some publications,[2,3] and it is also not possible to estimate the public health risks that humans can encounter by sharing the same environment with animals, handling animals, animal wastes, or consuming animal products in these countries. Therefore, the aim of this study was to estimate the extent of AR in intestinal indicator bacteria in randomly collected fecal samples from humans and selected food animals in Tanzania.

Materials and Methods

Sample sources and sampling

Samples were collected from two regions, Morogoro and Dar-Es-salaam, in Tanzania. The two regions were purposely selected because livestock keeping is intense due to urban and peri-urban market demand of livestock products. In each region a district (Morogoro and Kinondoni, respectively) was selected for sampling, and from each of these districts five wards were randomly selected as the sampling areas.

Freshly voided fecal materials (3–5 g) were collected in 2011–2013 from randomly picked commercial pigs ($n=97$), dairy ($n=97$), beef ($n=40$) cattle, and chicken farms ($n=97$). One animal was sampled per farm. In addition, indigenous chickens, which are widespread over a large geographical area in Tanzania and kept in extensive free-range systems, were sampled from nonpurposely identified households ($n=97$). In addition, 5–10 g of fecal samples were collected from humans ($n=97$) who were epidemiologically independent from each other. After collection, samples were stored in sterile containers at $\sim$4°C during transportation to the laboratory, where processing for bacteriological analyses was carried out immediately.

Enumeration of antibiotic-resistant indicator bacteria

Quantification of coliforms and *Enterococcus* spp. in feces was performed as previously described.[21] Briefly, 10-fold dilutions of fecal material were plated on MacConkey agar (Oxoid, Basingstoke, United Kingdom) plates without antibiotics and on plates with 2 mg/L CTX or 16 mg/L tetracycline (TE) or 16 mg/L ampicillin (AMP) or 256 mg/L sulfamethoxazole (STX), as well as on Slanetz–Bartley agar plates (Oxoid) without antibiotics and plates with 16 mg/L AMP or 16 mg/L vancomycin (VA). Concentrations of antibiotics were chosen to obtain a good separation between resistant and sensitive isolates based on wild-type minimum inhibitory concentration distributions for Enterobacteriaceae and *E. faecium* in EUCAST, as previously described.[21] Following overnight (coliforms) or 48 h (enterococci) incubation at 37°C, typical colonies were counted, and weighted average of colony forming units (CFU)/g of feces was determined. For statistical analysis of average CFU, a value of 1 CFU/g was given to samples with a concentration below the detection level.

Bacterial species identification

Presumptive *E. coli* from MacConkey agar plates were confirmed to species level using Gram stain and the IMViC phenotypic tests (indole, methyl red, Voges Proskauer, and citrate) as previously described.[22] Species identity of presumptive *Enterococcus* spp. colonies was determined by multiplex PCR.[23] Matrix assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (VITEK MS RUO; bioMérieux, France) was further used to identify presumptive non *E. coli* coliforms showing an ESBL phenotype and to identify *E. coli* and *Enterococcus* spp. isolates that did not show typical reactions by biochemical characterization or by multiplex PCR.

Antibiotic sensitivity testing

The disc diffusion method was used to determine phenotypic resistance in randomly picked, purified, and speciated isolates of *E. coli* from MacConkey agar and *Enterococcus* spp. isolates from Slanetz–Bartley plates without antibiotics, according to Clinical and Laboratory Standards Institute (CLSI)[24] guidelines, as previously described.[15] The following discs were used (*E. coli*): 5:1 STX (25 μg), AMP (10 μg), amoxicillin–clavulanic acid 2:1 (30 μg), gentamicin (CN, 10 μg), TE (30 μg), ciprofloxacin (CIP, 5 μg), streptomycin (10 μg), CTX (30 μg), and *Enterococcus* spp.: VA (30 μg), erythromycin (15 μg), CIP (5 μg), AMP (10 μg), chloramphenicol (30 μg), rifampicin (RD, 5 μg), CN (120 μg), and TE (30 μg). The reference strains *E. coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 were used for quality control.

The results were interpreted as Resistant, Intermediate, and Susceptible as indicated in the CLSI guidelines.[24] In this study multiple resistances were defined as a single bacterium being resisted three and more antimicrobials.[25]

Detection of ESBL phenotype and $bla_{CTX\text{-}M}$ *in putative ESBL-producing bacteria*

Colonies of *E. coli*, as well as lactose negative colonies, growing on MacConkey plates with added CTX were tested for synergy between CTX and clavulanic acid according to Lewis et al.,[26] and presence/absence of bla_{CTXM} was determined by PCR method according to Hasman *et al.*[27] *E. coli* ATCC strain 25922 and *Klebsiella pneumoniae* ATCC strain 700603 (HiMedia, Mumbai, India) were used as negative and positive controls for ESBL production test, respectively.[28]

Quantification of sulII *and* tetW *genes in fecal samples*

Gene copy numbers of *sulII* and *tetW* genes, as 16s RNA gene copies, were determined to estimate general levels of AR genes by a culture independent method. Details on DNA purification, the qPCR reactions, generation of standard curves, quality control of the performance of the qPCR reactions, and the primer sequences can be seen in Katakweba

et al.[21] The prediction interval of the number of amplicons was calculated using the formulas developed by Danzer and Currie.[29]

Statistical analysis

Statistical differences in mean ± standard error of the mean Log_{10} CFU/g feces and in Log_{10} number of *sulII* and *tetW* genes by qPCR were analyzed using one-way analysis of variance followed by Duncan's multiple range test. Binary logistic regression models (SPSS; version 16.0) and GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA) were used for the analysis of qualitative susceptibility data from disk diffusion test by chi-square and Fisher's exact test.

Ethical clearance

Ethical approval was obtained from National Institute for Medical Research of Tanzania (NIMR) (reference no. NIMR/HQ/R.8a/Vol.IX/936 to the first author), and fecal samples were obtained from humans with written consent from the participants.

Results

Bacterial isolates picked for detailed characterization

In the current study, estimation of resistance levels was based on counting of bacteria growing on selective agar media with and without added antimicrobials and on characterization of purified and speciated bacteria. With regard to the latter, all lactose positive >0.5 mm colonies from MacConkey agar were shown to *E. coli*. Fifty-nine non-lactose fermenters were picked, because they were observed to grow on MacConkey agar plates containing CTX. These were identified by MALDI-TOF MS to be *Klebsiella pneumonia* ($n = 49$), *Ochrobactrum* spp. ($n = 3$), *Achromobacter* spp. ($n = 1$), and lactose negative *E. coli* ($n = 3$). Three isolates remained unidentified. A random subset of 164 presumptive Enterococcus colonies from Slanetz–Bartley agar was characterized. The species distribution according to origin of the sample is shown in Table 1. The majority of isolates from all animal species and humans were *E. faecium* (92%) followed by *E. hirae* and *E. faecalis*.

Table 1. Species Composition of *Enterococcus* Isolates from Human and Food Animals in Tanzania

Sample sources	E. faecium	E. faecalis	E. hirae	*Total*
Human	33	1	3	37
Exotic chickens	27	0	1	28
Indigenous chickens	17	2	0	19
Dairy cattle	41	1	2	44
Beef cattle	13	0	0	13
Pigs	20	1	2	23
Total	151 (92.07)	5 (3.04)	8 (4.09)	164

Randomly picked, presumptive colonies of *Enterococcus* on Slanetz–Bartley agar were subjected to multiplex PCR for species identification. Matrix assisted laser desorption ionization–time of flight mass spectrometry was further used to identify colonies that did not show typical reactions by this PCR.

Quantification of resistant bacteria in fecal samples from humans and food animals

The number of antimicrobial resistant coliforms in fecal samples was presented as Log_{10} CFU/g, as well as the proportion of samples that showed growth over the detection limit. Table 2 shows results obtained when quantifying coliform bacteria.

More than 70% of all samples contained coliforms that were resistant to TE, STX, or AMP, and coliforms that were resistant to CTX were observed in 40% of all samples. Significantly higher numbers of samples were positive with resistant colonies among dairy cattle compared with beef cattle, irrespective of antimicrobial agents. The total number of coliforms per gram feces was between Log_{10} 4.0 and log_{10} 5.1. The observed number of bacteria was significantly lower in beef cattle than in other animals and in humans (Table 2). With the exception of beef cattle, CTX-resistant coliforms were observed in >30% of samples of all species, however, with relatively low numbers per animal. On average, CFU of CTX-resistant coliforms in samples from positive humans were 2.2 Log_{10} CFU/g (Table 2); however, up to 7.12 Log_{10} CFU/g were detected in individual samples (data not shown).

As shown in Table 2, the total number of enterococci from beef cattle was low compared to other animal species. AMP and VA-resistant enterococci were detected in samples from humans and all animal species, although with low frequency (16% of human and 5.3% of all samples, respectively) (Table 2). In samples, counts of AMP resistant colonies ranged from 2.80 to 6.95 Log_{10} CFU/g, and counts of VA-resistant enterococci ranged between 3.75 and 6.34 Log_{10} CFU/g.

When the number of resistant bacteria was expressed relative to total number of coliform bacteria and *Enterococcus* spp. (Log_{10} resistant colonies/Log_{10} total number of colonies), it was generally found that beef cattle had significantly lower proportion of resistant bacteria compared to other sample sources (Supplementary Tables S1 and S2; Supplementary Data are available online at www.liebertpub.com/mdr). Furthermore, all samples from other sources than beef cattle had high proportions of AMP-, STX-, and TE-resistant coliforms. The highest proportions of AMP-resistant *Enterococcus* spp. were observed among humans and indigenous chicken, while the highest proportion of VA-resistant enterococci was observed in samples from pigs.

Phenotypic resistance in single isolates of indicator bacteria

Isolates for characterization of resistance by disc diffusion were obtained from MacConkey and Slanetz–Bartley agar without antibiotics, and results for *E. coli* are listed in Table 3. The prevalence of *E. coli* resistant to TE, STX, and AMP was high (68.8%, 61.1%, and 54.8%). Resistance to CTX was demonstrated in 36.4% of all isolates, with more than 50% of the isolates from dairy cattle showing this resistance. As above, there was a large difference between beef and dairy cattle. CIP resistance was by far most widespread among chicken isolates (>50%).

TABLE 2. QUANTIFICATION (Log_{10} COLONY FORMING UNITS/G ± STANDARD ERROR) OF ANTIMICROBIAL RESISTANT *ESCHERICHIA COLI* AND *ENTEROCOCCUS* SPP. PROPORTION (*N*) OF SAMPLES (*N*) HARBORING RESISTANCE FROM HUMANS AND FOOD ANIMALS

	E. coli					Enterococcus *spp.*		
Sample source (*N*)	*Without antimicrobial* (n/N)[#]	*AMP* (n/N)	*CTX* (n/N)	*STX* (n/N)	*TE* (n/N)	*Without antimicrobial* (N/N)[#]	*AMP* (n/N)	*VA* (n/N)
Human (97)	5.0 ± 0.1^{a} (97/97)	3.9 ± 0.2^{a} (81/97)	2.2 ± 0.2^{a} (47/97)	3.6 ± 0.2^{a} (79/97)	3.4 ± 0.2^{ba} (77/97)	4.30 ± 0.13^{b} (97/97)	0.94 ± 0.16^{a} (20/97)	0.39 ± 0.11^{a} (1/97)
Exotic chicken (97)	4.9 ± 0.1^{a} (97/97)	3.4 ± 0.2^{ba} (75/97)	1.4 ± 0.2^{b} (32/97)	3.4 ± 0.2^{a} (74/97)	3.1 ± 0.2^{b} (70/97)	4.86 ± 0.13^{a} (79/97)	0.56 ± 0.16^{ba} (12/97)	0.40 ± 0.11^{a} (8/97)
Indigenous chicken (97)	5.1 ± 0.1^{a} (97/97)	3.2 ± 0.2^{b} (71/97)	1.9 ± 0.2^{ba} (40/97)	3.8 ± 0.2^{a} (78/97)	3.8 ± 0.2^{a} (87/97)	4.56 ± 0.13^{ba} (97/97)	0.69 ± 0.16^{ba} (17/97)	0.28 ± 0.11^{a} (7/97)
Dairy cattle (97)	4.8 ± 0.1^{a} (97/97)	3.0 ± 0.2^{b} (80/97)	2.3 ± 0.2^{a} (48/97)	3.5 ± 0.2^{a} (80/97)	3.0 ± 0.2^{b} (83/97)	4.72 ± 0.13^{ba} (97/97)	0.82 ± 0.16^{a} (21/97)	0.20 ± 0.11^{ba} (5/97)
Beef cattle (40)	4.4 ± 0.2^{b} (40/40)	1.3 ± 0.3^{c} (18/40)	0.2 ± 0.3^{c} (3/40)	1.2 ± 0.3^{b} (20/40)	1.4 ± 0.3^{c} (15/40)	3.67 ± 0.2^{c} (40/40)	0.23 ± 0.25^{b} (3/40)	0.17 ± 0.16^{ba} (2/40)
Pig (97)	4.8 ± 0.1^{a} (97/97)	3.1 ± 0.2^{b} (68/97)	1.8 ± 0.2^{ba} (40/97)	3.5 ± 0.2^{ba} (77/97)	3.4 ± 0.2^{ba} (79/97)	4.52 ± 0.13^{ba} (97/97)	0.40 ± 0.16^{ba} (11/97)	0.26 ± 0.11^{a} (5/97)

E. coli: [abc]Mean Log_{10} CFU/g within each column bearing same letter are not significantly different at $p < 0.05$.
#, numbers in brackets indicate the proportion (*n*) of samples harboring resistant *E. coli* above the detection level of 100 CFU/g out of the total number of samples (*N*) tested. CFU were determined by spreading 10-fold diluted fecal samples on MacConkey agar plates without antimicrobials (total coliform counts) or with antimicrobials at the following concentrations: AMP (16 mg/L), CTX (2 mg/L), STX (256 mg/L), TE (16 mg/L).
Enterococcus spp.: [abc]Mean Log_{10} CFU/g within each column bearing same letter are not significantly different at $p < 0.05$.
#, numbers in brackets indicate the proportion (*n*) of samples harboring resistant *Enterococcus* above the detection level of 100 CFU/g out of the total number of samples (*N*) tested. CFU were determined by spreading 10-fold diluted fecal samples on Slanetz–Bartley agar without antimicrobials (total enterococci counts) or with AMP (16 mg/L) or VA (16 mg/L) added to the plates.
CFU, colony forming units; AMP, ampicillin; CTX, cefotaxime; STX, sulfamethoxazole; TE, tetracycline; VA, vancomycin.

Isolates of enterococci showed high levels of resistance toward RD (75.2%) and a much lower level of resistance to E (15%) and TE (13.8%). Resistance toward CN (120 μg) was not observed. Notably, Vancomycin-Resistant Enterococci (VRE) were present in humans, pigs, dairy cattle, and chickens, and AMP resistance was observed at high levels in cattle isolates (Table 4).

Numbers of multiresistant E. coli *and enterococci*

Among the *E. coli* isolates, multiresistance was observed in 254 (65.1%) of the isolates tested. The number of different resistances in strains obtained from different hosts is shown in Supplementary Table S3. Two isolates showed resistance to all eight antimicrobials tested. For *Enterococcus* spp., multiresistance was observed in 18 (7.5%) of all the tested isolates. The maximum resistance was observed up to four antimicrobials. The number of resistances observed per strain is shown in Supplementary Table S4.

Detection of $bla_{CTX\text{-}M}$ *genes in presumptive ESBL isolates*

Escherichia coli showing resistance to CTX and lactose negative colonies growing on MacConkey agar with CTX were analyzed, and all these showed synergy when cefotaxime and clavulanic acid were tested together (>5 mm increase in zone diameters). Further they were all positive for $bla_{CTX\text{-}M}$ by PCR (Supplementary Fig. S1).

Quantification of sulII *and* tet(W) *genes by qPCR*

The highest numbers of *sulII* gene copies were observed in cattle (Log_{10} 5.68) and the lowest in pigs (Log_{10} 5.37), but there were no significant ($p > 0.05$) differences between sample sources (Table 5). For *tetW* genes, there was a significant difference in sample sources, and cattle had highest gene copy number (Log_{10} 7.39), while the lowest number was observed in humans (Log_{10} 6.41). 16s rDNA copy numbers were significantly different between sample sources, indicating that the total numbers of bacteria per gram feces were different between species. When expressed relatively to 16s rRNA gene copies, only cattle was significantly different from the other animals/humans with regard to both genes analyzed.

Discussion

Three different techniques were used in this study for assessment of the general levels of resistance in randomly collected fecal samples from humans and food animals in Tanzania. Two of the methods were linked, since they were both based on bacteria growing on selective media, while the third qPCR assessed resistance levels by a growth independent method. A recent study, which quantified gene copies of 14 antimicrobial resistance-genes by qPCR and compared gene number of resistance genes to Log_{10} CFU coliform with phenotypic resistance in pig feces, concluded that the two measurements had no statistical association. However, qPCR was shown to give a good representation for the general resistance level and was less influenced by sample bias.[30] Thus our approach assured that results obtained would reveal differences between the various hosts based on biologically different indicator systems. This must

Table 3. Antimicrobial Susceptibility Testing of *E. coli* from Humans and Food Animals in Tanzania

Antimicrobials	*Isolate status*	*Humans (N = 70)*	*Indigenous chickens (N = 70)*	*Exotic chickens (N = 70)*	*Pigs (N = 70)*	*Dairy cattle (N = 70)*	*Beef cattle (N = 40)*	*Total (N = 390)*	p-*Value*
STX	R	48 (68.6)	54 (77.2)	42 (60)	42 (60)	23 (32.9)	21 (52.5)	230 (59)	0.0001
	I/S	22 (31.4)	16 (22.8)	28 (40)	28 (40)	47 (67.1)	19 (47.5)	187 (41)	
CN	R	7 (10)	5 (7.1)	10 (14.3)	4 (5.7)	13 (18.6)	16 (15)	45 (11.5)	0.0007
	I/S	63 (90)	65 (92.9)	60 (85.7)	66 (94.3)	57 (81.4)	24 (85)	345 (88.5)	
CIP	R	13 (18.6)	37 (52.9)	36 (51.4)	7 (10)	12 (17.1)	11 (27.5)	116 (29.7)	0.0001
	I/S	57 (81.4)	33 (47.1)	34 (48.6)	63 (90)	58 (82.9)	29 (72.5)	274 (70.3)	
AMP	R	40 (57.1)	48 (68.6)	55 (78.6)	27 (38.6)	18 (25.7)	15 (37.5)	203 (52.1)	0.0001
	I/S	30 (42.9)	22 (31.4)	15 (21.4)	43 (61.4)	52 (74.3)	25 (62.5)	187 (47.9)	
CTX	R	22 (31.4)	22 (31.4)	32 (45.7)	17 (24.3)	40 (57.1)	9 (22.5)	142 (36.4)	0.0001
	I/S	48 (68.6)	48 (68.6)	38 (54.3)	53 (75.7)	30 (42.9)	31 (77.5)	248 (63.6)	
S	R	39 (55.7)	49 (70)	40 (57.1	35 (50)	35 (50)	16 (40)	214 (54.9)	0.0002
	I/S	31 (44.3)	21 (30)	30 (24.9)	35 (50)	35 (50)	24 (60)	176 (45.1)	
TE	R	50 (71.4)	54 (77.1)	51 (72.9)	51 (72.9)	27 (38.6)	26 (65)	259 (66.4)	0.0002
	I/S	20 (28.6)	16 (22.9)	19 (27.1)	19 (27.1)	43 (61.4)	14 (35)	131 (33.6)	
MC	R	10 (14.3)	18 (25.7)	7 (10)	4 (5.7)	17 (24.3)	1 (2.5)	57 (14.6)	0.0001
	I/S	60 (85.7)	52 (74.3)	63 (90)	66 (94.3)	53 (75.7)	39 (97.5)	333 (85.4)	

STX/trimethoprim 5:1 (STX, 25 μg), AMP (10 μg), amoxycillin–clavulanic acid 2:1 (AMC, 30 μg), gentamicin (CN, 10 μg), ciprofloxacin (CIP, 5 μg), TE (30 μg), streptomycin (S, 10 μg), and CTX (30 μg). One randomly picked *E. coli* colony was obtained from fecal samples (*N*) of human and food animals, and susceptibility to the shown antimicrobials was determined by disc diffusion test according to CLSI standards. The *p*-value is for the nil hypotheses that the frequency of resistant isolates was not different between the different human and food animal species tested.

R, resistant; I, intermediate; S, susceptible; CLSI, Clinical and Laboratory Standards Institute.

be considered the strength of the study, as previously pointed out by others.[31–33]

Apart from studies focusing on the contamination of animal products like meat and milk,[34,35] there are no data in Tanzania reporting on number of coliforms and enterococci and their AR profiles from food animals. The present study showed that beef cattle contained lower number resistant coliforms and enterococci than dairy cattle, pigs, and intensively kept exotic chickens, as well as local chickens. These findings corroborate those of Hershberger *et al.*,[36]

Table 4. Antimicrobial Susceptibility Testing of *Enterococcus* spp. from Humans and Food Animals in Tanzania

Antibiotics	*Isolate status*	*Human (N = 40)*	*Indigenous chicken (N = 40)*	*Exotic chicken (N = 40)*	*Pig (N = 40)*	*Dairy cattle (N = 40)*	*Beef cattle (N = 40)*	*Total (N = 240)*	p *Value*
E	R	9 (22.5)	6 (15)	6 (15)	6 (15)	3 (7.5)	6 (15)	36 (15)	0.0005
	I/S	31 (72.5)	34 (85)	34 (85)	34 (85)	37 (92.5)	34 (85)	204 (85)	
RD	R	30 (75)	33 (82.5)	33 (82.5)	28 (70)	27 (67.5)	23 (57.5)	174 (75.2)	0.0984
	I/S	10 (25)	7 (17.5)	7 (17.5)	12 (30)	13 (32.5)	17 (42.5)	66 (27.5)	
VA	R	1 (2.5)	5 (12.5)	0	3 (7.5)	0	3 (7.5)	12 (5)	0.0704
	I/S	39 (97.5)	35 (87.5)	40 (100)	37 (92.5)	40 (100)	37 (92.5)	228 (95)	
TE	R	5 (12.5)	10 (25)	1 (2.5)	4 (10)	3 (7.5)	10 (25)	33 (13.8)	0.012
	I/S	35 (87.5)	30 (75)	39 (97.5)	36 (90)	37 (92.5)	30 (75)	207 (86.3)	
CIP	R	0	6 (15)	3 (7.5)	0	0	0	9 (3.8)	0.0006
	I/S	40 (100)	34 (85)	37 (92.5)	40 (100)	40 (100)	40 (100)	231 (96.2)	
CN	S	40 (100)	40 (100)	40 (100)	40 (100)	40 (100)	40 (100)	40 (100)	1
C	R	0	4 (10)	0	0	1 (2.5)	0	5 (2.1)	0.0077
	I/S	40 (100)	36 (90)	40 (100)	40 (100)	39 (97.5)	40 (100)	235 (97.9)	
AMP	R	0	2 (5)	0	1 (2.5)	0	9 (22.5)	12 (5)	0.0001
	I/S	40 (100)	38 (95)	40 (100)	39 (97.5)	40 (100)	31 (77.5)	228 (95)	

VA (30 μg), erythromycin (E, 15 μg), CIP (5 μg), AMP (10 μg), chloramphenicol (C, 30 μg), rifampicin (RD 5 μg), gentamicin (CN, 120 μg), and TE (30 μg). Randomly picked colonies of *Enterococcus* from Slanetz–Bartley agar plates without antimicrobials were subjected to disc diffusion test according to CLSI standards. The *p*-value shown is for the nil hypothesis that the frequency of resistant isolates was not different between the different human and food animal species tested.

R, resistant; I, intermediate; S, susceptible.

Table 5. Least Square Mean (±Standard Error) of Log_{10} Copies of *sulII* and *tetW* Genes Per Gram Feces and Number of Resistance Gene Amplicons as Percent of Total 16s rRNA Gene Copies in Fecal Samples from Humans and Food Animals in Tanzania

Animal species	N	sulII *(% RGA/16s rRNA)*	tetW *(% RGA/16s rRNA)*	*16s rRNA*
Human	22	5.36 ± 0.10^{a} (0.002 ± 0.10^{b})	6.41 ± 0.17^{c} (0.059 ± 1.88^{b})	10.15 ± 0.16^{a}
Exotic chickens	22	5.44 ± 0.10^{a} (0.020 ± 0.10^{b})	6.55 ± 0.17^{cb} (0.980 ± 1.88^{b})	9.84 ± 0.16^{b}
Indigenous chickens	22	5.44 ± 0.10^{a} (0.002 ± 0.10^{b})	6.53 ± 0.18^{cb} (2.149 ± 2.00^{b})	9.33 ± 0.17^{c}
Cattle	22	5.69 ± 0.10^{a} (0.343 ± 0.10^{a})	7.39 ± 0.17^{a} (11.881 ± 1.88^{a})	9.58 ± 0.16^{cb}
Pigs	22	5.37 ± 0.10^{a} (2.389 ± 1.88^{b})	6.97 ± 0.17^{ba} (0.002 ± 0.10^{b})	9.61 ± 0.16^{cb}

[abc]Mean Log_{10} RGA within each column bearing same letter are not significantly different at $p < 0.05$. qPCR reactions were performed using Stratagene Mx 3000p PCR cycler with Maxima SYBR Green/ROX qPCR mix. Standard curves of the respective genes were used for calculation of the gene copy number per gram of sample.

RGA, number of resistance gene amplicons.

who showed that enterococci from dairy cattle in United States of America had higher percentage of resistant bacteria compared to those of beef cattle. Mastitis is a common disease in dairy cattle, and antimicrobials are commonly used for treatments,[37,38] and we presume that treatment of this disease is a main driver of resistance level in dairy cows in Tanzania.

It was unexpected that the numbers of resistant coliforms and enterococci in local chickens were higher than in intensively kept exotic chickens, since local chickens are kept in extensive way, left to scavenge for food in villages. Such birds are rarely treated with antimicrobials.[14] It is possible that these results are because of exposure to compounded feed and antimicrobials used in other livestock, but it may also be a result of the low hygiene in villages in Tanzania. Birds may come into contact with human feces while scavenging for food and, thus, be colonized with resistant bacteria from humans.

Cephalosporin drugs are not widely available for veterinary use and are rarely used in small scale in farms in Tanzania.[14] Surprisingly, the present study showed widespread occurrence of resistance to cephalosporins in coliforms and *E. coli* from farm animals. In the isolates that were characterized by PCR, this was found to be due to presence of ESBL of the $bla_{CTX\text{-}M}$-type. This can either be due to co-selection of ESBLs by other antimicrobials used[13] or it can indicate spread of bacteria from humans, where there is widespread use of cephalosporin. The most likely transmission direction for resistant bacteria in developed countries is believed to be from animals to man. Our results indicate that this may be different in extensive production systems in poor countries. Whether this is associated to poor sanitary conditions in rural areas, exposing animals to fecal contamination from humans[39,40] or drug residues in feed and the environment, remains to be investigated.

A survey on veterinary antimicrobials use in Tanzania found that TE, AMP, and sulfonamides were the most commonly used drugs in farm animals.[14] In the present study resistance to these drugs was predominant in *E. coli* isolates from cattle, pigs, and chickens. These drugs are commonly used in livestock production in Sub-Saharan Africa,[41,43] and resistant bacteria with these resistances are also seen outside Tanzania.[42] The results further showed that multiple resistance is common among *E. coli*, but less so in enterococci, from animals and humans in Tanzania.

The finding with regard to enterococci is in contrast with those of Byarugaba *et al.*[44] who reported that 35 and 46 percent of enterococci spp. and *E. coli* from food of animal origin in Uganda were resistant to five or more antimicrobials. Also Tellevik *et al.*,[16] reported high prevalence of multidrug resistance (94%) among *E. coli* and *K. pneumonia* from children in Dar es Salaam, Tanzania. Compared to findings by EFSA,[8] levels of AR in coliform indicator bacteria from animals and humans in Tanzania are higher than in the European countries with intensive livestock production systems. There are currently no reliable data on antimicrobial consumption in livestock in Tanzania, but the data on resistance levels may indicate that consumption of some antimicrobials is high.

Resistance levels in *Enterococcus* spp. from Tanzania were comparable or lower than those reported from Europe,[8,45] with the exception of the high levels of resistance to RD. This drug is only used for humans in Tanzania, and these results, too, may suggest transfer of bacteria from humans to animals, for example, through environmental contamination. However, with the current situation in developing countries, where use of antimicrobials is not stringently controlled, the use of human drugs to treat animal diseases is also a possibility.[46,47] Resistance to VA continued to be detected, although at very low levels in enterococcal isolates from animals in Europe.[8]

Avoparcin, a glycopeptide showing cross-resistance to medically important glycopeptides, has been used in the European Community as a growth promoter in animal feeds, and this has been documented to select VRE in studies using molecular analysis of isolates of *E. faecium*.[48,49] This growth promoter has not been used in Tanzania. It was therefore surprising to find VRE among isolates obtained from animal species. The VRE in human-associated samples may be a result of VA use in hospitals,[50] and it is recommended to analyze more in detail for the presence of VRE in the human population in Tanzania, as resistance seen in this study implies an uncontrolled presence of VRE in the population.

ESBL producing bacteria constitute a particularly threatening human health problem, for which a food animal source may be present.[51,52] The current study presents the first report on the presence of ESBL in Tanzania from food animals. The CTX-M type is currently the most widespread and threatening ESBL, particularly in community-acquired infections.[53,54] *E. coli* and *K. pneumoniae* were found to be the most common carriers of ESBL genes. These species were also dominant ESBL producers among patients attending a tertiary hospital in northern Tanzania.[13] ESBL producing bacteria have been also reported in studies from Uganda,[55] East Africa in general,[56] and Central Africa.[57] In the current study, a $bla_{CTX\text{-}M}$ PCR

method was used, and no further subdivision was attempted. We cannot rule out that some of the isolates may carry additional ESBL genes, as it has been reported that several genes can be present in the same bacteria.[58] Mshana *et al.*[15] have previously reported the presence of $bla_{\text{CTX-M-15}}$ in Tanzania. The gene was found mostly to be located on transferrable IncY and IncF plasmids, suggesting a high possibility for transfer.

Tellevik *et al.*[16] studied the prevalence of ESBL bacteria, showing a higher prevalence among hospitalized (50.4%) than community children (11.6%). Apart from detecting the $bla_{\text{CTX-M-15}}$ gene, this study reported the presence of plasmid-mediated AmpC beta-lactamase CMY-2 in Tanzania. Over and above, ESBL prevalence was significantly higher among HIV positive (89.7%) than HIV negative (16.9%) children. A review on ESBL in East African hospitals published by Sonda *et al.*,[59] reported CTX-M, TEM, CTX-M-15, CMY-2, AmpC, and OXA-48 as the most frequent ESBL genes, while KPC and NDM were infrequent. Regular surveillances are needed in different countries to aid in understanding of the epidemiology of these genes, and in relation to the current study, it would be relevant to include surveillance for genes encoding resistance to aztreonam and carbapenemases, as well as colistin.

The choice of genes to be included in the culture independent approach was justified by two factors. The *sulII* is the most common gene encoding sulfonamide resistance in *E. coli*,[60] and *tetW* is a ribosomal protection type of TE resistance genes,[61] which is the most common TE resistance gene in human fecal samples.[62] Furthermore, STX and TE were the most commonly used antimicrobials in the study area,[14] hence the possibility of detecting these genes was high. Surprisingly, the differences seen by characterization of coliforms and by disc diffusion, that is, that humans generally carried the highest levels of resistance could not be confirmed by this approach. In this study, only cattle were different from the other groups, and only with respect to copy numbers of *tetW*. This result confirms previous observation that culture dependent and culture independent characterization of resistance levels do not have statistical significant agreement,[30] and further studies are needed to understand how to interpret fluctuations in AMR gene pools, as determined by culture independent approaches.

In conclusion, this article has quantified the levels of resistance in commensal coliforms, *E. coli* and enterococci, from healthy animals and humans in Tanzania. It has shown that resistance level for several antimicrobials was higher in isolates from humans than from food animals and also that resistance levels in coliforms from food animals were higher than those reported from Europe; especially resistance to TE, sulfonamides, and AMP resistance, the most common human and veterinary antimicrobials in Tanzania,[60] was high. The antimicrobial susceptibility data from especially enterococci indicated that humans may be a possible source of AR bacteria in animals. The study reports for the first time prevalence of ESBL bacteria in food animals in Tanzania, and it documents the presence of VRE in these animals. Despite its exploring nature, the study provides new insights into AR, and it points to a need for further research into the dynamics of transmission of AR among animals, people, and environment. Further studies are also recommended to explore pathways of sharing bacteria between human and animals.

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