Journal of Food Protection, Vol. 79, No. 9, 2016, Pages 1635–1641
doi:10.4315/0362-028X.JFP-15-573

Research Note

Load and Prevalence of Antimicrobial-Resistant *Escherichia coli* from Fresh Goat Meat in Arusha, Tanzania

GASPARY MWANYIKA,[1] DOUGLAS R. CALL,[1,2] BENARDETHER RUGUMISA,[1] CATHERINE LUANDA,[1] REHEMA MURUTU,[1] MURUGAN SUBBIAH,[2] AND JORAM BUZA[1]*

[1]*Department of Health and Biomedical Sciences, School of Life Science and Bioengineering, Nelson Mandela African Institution of Science and Technology (NM-AIST), Arusha, Tanzania; and* [2]*Paul G. Allen School for Global Animal Health, Washington State University, Pullman, Washington 99164, USA*

MS 15-573: Received 14 December 2015/Accepted 29 April 2016

ABSTRACT

Given the potential public health risks associated with a burgeoning goat meat industry in Tanzania, we estimated the load of *Escherichia coli* and the prevalence of antibiotic-resistant strains for goat meat by using a cross-sectional study design (June to July 2015). Five large ($n = 60$ samples) and five small ($n = 64$ samples) slaughterhouses were sampled over a period of four to six visits each. Meat rinsate was prepared and plated onto MacConkey agar, and presumptive *E. coli* colonies were enumerated and reported as CFU per milliliter of rinsate. In total, 2,736 presumptive *E. coli* isolates were tested for antibiotic drug sensitivity by using breakpoint assays against 11 medically important antibiotics. *E. coli* was recovered from almost all the samples (96.8%), with counts ranging from 2 to 4 log CFU ml^{-1}, and there was no significant difference ($P = 0.43$) in recovery according to facility size (average, 3.37 versus 3.13 log CFU ml^{-1}, large and small, respectively). Samples from large facilities had relatively higher prevalence ($P = 0.026$) of antibiotic-resistant *E. coli* compared with small facilities. This was mostly explained by more ampicillin (30.1 versus 12.8%) and amoxicillin (17.6 versus 4.5%) resistance for large versus small facilities, respectively, and more tetracycline resistance for small facilities (5.6 versus 10.6%, respectively). Large slaughter operations may serve as foci for dissemination of antibiotic-resistant bacteria via food products. More effective hygiene practices during slaughter and meat handling would limit the probability of transmitting antibiotic-resistant *E. coli* in goat meat.

Key words: Antimicrobial resistance; Arusha; *Escherichia coli*; Goat meat; Tanzania

Animal meat is an important protein source worldwide. In some countries, goats are an important source of meat, but they can also serve as carriers of pathogenic *Escherichia coli, Salmonella enterica* (mostly serovars Typhimurium and Enteritidis), and *Campylobacter* spp. *(10, 16)*. These pathogens can spread to people through contact with the animals or their excreta, or by handling and consuming raw or undercooked contaminated meat *(23)*. Contaminated food products remain the major cause of foodborne illness in developing countries; in Southeast Asia and Africa, such contamination accounts for more than 1.46 million annual deaths in children <5 years old. Tanzania is one of the 15 developing countries that collectively bear 73% of these deaths *(5, 25)*.

E. coli is a common bacterial contaminant of meat, and most strains of *E. coli* are commensal enteric organisms *(33)*. Their presence in food products indicates direct or indirect fecal contamination that most likely occurs due to deficits in hygiene during product preparation. Although most commensal *E. coli* strains are harmless, there are many strains that are harmful to people *(13)*. Furthermore, commensal *E. coli* are thought to serve as reservoirs for antimicrobial resistance and associated antimicrobial resistance genes that can be shared with pathogens *(3, 26)*. Antibiotic resistance in pathogens can complicate treatment of infectious disease, leading to prolonged hospitalization, treatment failure, and death *(39)*. In Tanzania, the occurrence of antimicrobial-resistant (AMR) infections has been reported *(17)*.

In developing countries, the role of food animal products in transmitting AMR pathogens to people is underappreciated *(19)*. In Tanzania slaughter facilities in urban centers usually operate under regulatory oversight of official meat inspectors and health officers who evaluate meat quality, sanitation practices, and staff hygiene. In these facilities animal stock comes from local traders who purchase the animals either directly from local farmers or obtain animals from primary and secondary livestock markets found within and outside the urban zones. In rural areas, slaughter infrastructure is usually rudimentary and meat inspection and hygiene are scarce. Slaughter facilities in these areas obtain stock mainly from individual farmers and primary livestock markets *(28, 38)*. In Arusha, goat meat is a popular animal food product made famous as roasted meat (*nyama choma*). It is sold in fast-food stalls and bars commonly found in local municipalities. In these settings,

* Author for correspondence. Present address: P.O. Box 447, Arusha, Tanzania. Tel: +255688012616; Fax: +255 (27) 2970016; E-mail: joram.buza@nm-aist.ac.tz.

poor sanitary practices, including procedures used in slaughter, are common and can increase the transmission of foodborne pathogens and AMR bacteria to people *(25)*.

To assess the levels of *E. coli* load as a measure of fecal contamination and estimate the prevalence of AMR *E. coli* in goat meat samples from selected slaughterhouses in the Arusha area, we grouped the slaughterhouses as large (10 to 150 goat capacity per day) and small (1 to 3 goat capacity per day) slaughter operations.

MATERIALS AND METHODS

Sampling collection and processing. Between June and July 2015, two or three fresh meat samples (250 g each) were purchased weekly from both large (five premises: A, B, C, D, and E; $n = 60$ samples, 12 per premise) and small (five premises: F, G, H, I, and J; $n = 64$ samples, 12 to 13 per premise) slaughter facilities. Samples were stored separately in clearly labeled, sterile polyethylene bags and carried to the laboratory in an ice-cold box within 2 h of collection. In the laboratory, subsamples (25 g each) were washed thoroughly with 25 ml of double-distilled water by soaking and vigorously shaking the subsamples in sterile plastic bags. An aliquot (1 ml per sample) was transferred into a 2-ml microcentrifuge tube containing glycerol (15% final concentration) and stored at −20°C as a source for additional dilutions if needed. The remaining portion of the meat wash was transferred into a 15-ml Falcon tube (BD, Franklin Lakes, NJ) for bacterial counts on the same day of collection.

***E. coli* isolation and enumeration.** Meat rinsate was analyzed for *E. coli* counts by procedures described previously *(31)*, with minor modifications to improve the *E. coli* detection limit. Modifications included an increase in the size of product sample (increased from 10 to 25 g to 100 to 250 g), and meat rinsate was obtained by diluting the samples to a ratio of 1:1 (25 g in 25 ml of sterile double-distilled water) compared with the previously used ratio of 1:10 (10 g to 90 ml of sterile double-distilled water). MacConkey agar (Difco, BD, Sparks, MD) was prepared according to the manufacturer's specifications and poured into petri plates (60 by 15 mm). Meat rinsate was serially diluted (10-fold, 0 to 10^{-3}), and 100 μl of each dilution was spread plated directly onto the agar plates by using sterile glass beads. The plates were briefly dried at room temperature and incubated overnight at 37°C. After incubation the plates were checked for the presence of presumptive *E. coli* colonies and counted. *E. coli* typically appears as a dry colony that is pink to rose-red on MacConkey agar. The number of CFU per milliliter of bacteria from the meat wash was then calculated *(36)*. From each sample 24 presumptive *E. coli* colonies were picked with sterile toothpicks and inoculated into 150 μl of Luria-Bertani broth (Difco, BD) contained within wells from a 96-well plate and incubated overnight at 37°C. After overnight incubation glycerol (15% final concentration) was added to each well in the 96-well plates containing *E. coli* culture, and the plates were stored at −80°C until further use.

Determination of antimicrobial susceptibility in *E. coli*. Before testing, the frozen 96-well culture plates were left to thaw for 20 to 30 min, and a duplicate working plate was prepared by using a sterile 96-pin replicator. All presumptive *E. coli* isolates were confirmed for their biochemical identity by transferring the cultures onto a chromogenic selective agar plate (Hi Media Laboratories Prt. Ltd., Mumbai, India) by using a 96-pin replicator. Bluish green colonies were identified as typical *E. coli,* and this procedure confirmed that 91.7% (1,320 isolates from small facilities) and 98% (1,416 isolates from large facilities) of the presumptive *E. coli* isolates were *E. coli*. To determine the prevalence of antibiotic-resistant *E. coli,* breakpoint assays for 11 antibiotics were used *(35)*. Antibiotic concentrations were guided by Clinical and Laboratory Standard Institute recommendations (*8*): ampicillin, 32 μg/ml (VWR International, Radnor, PA); amoxicillin, 32 μg/ml (MP Biomedicals, Santa Ana, CA); chloramphenical, 32 μg/ml (Mediatech, Inc., Manassas, VA); ciprofloxacin, 4 μg/ml (Enzo Life Sciences, Inc., Farmingdale, NY); ceftazidime, 8 μg/ml (Sigma, St. Louis, MO); cefotaxime, 4 μg/ml (Chem-Impex International, Inc., Wood Dale, IL); gentamicin, 16 μg/ml (Mediatech); streptomycin, 16 μg/ml (Amresco Inc., Solon, OH); sulfamethoxaxole, 512 μg/ml (MP Biomedicals); tetracycline, 16 μg/ml (MP Biomedicals); and trimethoprim, 8 μg/ml (MP Biomedicals).

The breakpoint assay was performed using MacConkey agar plates containing individual antibiotics. Approximately 1 to 2 μl ($\sim 10^4$ CFU per spot) of bacterial inoculum from each 96-well plate was transferred using a sterile 96-pin replicator and spotted onto the MacConkey agar plates containing an antibiotic. Plates were dried for ~15 min at room temperature and incubated overnight at 37°C. After incubation the plates were examined for the presence of resistant bacteria. Antibiotic resistance was evident when an isolate grew on an agar plate containing an antibiotic. *E. coli* K-12 was used as a negative control and *E. coli* NM-1 (resistant to ampicillin, ciprofloxacin, chloramphenicol, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim) and *E. coli* NM-2 (resistant to ampicillin, amoxicillin–clavulanic acid, ceftazidime, ciprofloxacin, kanamycin, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim) as a positive control. NM-1 and NM-2 were originally isolated from water sources in northern Tanzania. Antibiotic resistance phenotypes were characterized at the Nelson Mandela African Institution of Science and Technology (Arusha, Tanzania), and the genotypes were confirmed at Washington State University (Pullman) by using breakpoint assays as described here.

Statistical analysis. All counts in CFU per milliliter were log transformed at base 10 to satisfy assumptions of a normal distribution *(15)*. To estimate the difference of *E. coli* load in goat meat samples between large and small slaughter facilities, a two-sample Student's *t* test was used. Comparison of variance in prevalence of AMR *E. coli* between and within large and small slaughterhouses and among sampling sites across tested antibiotics was done by two-way analysis of variance to compute the *F* statistic and Tukey's honestly significant difference post hoc test (R software version 3.2.1; stats package). Results at $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Overall, 120 (96.8%) of 124 goat meat samples were positive for *E. coli,* with counts ranging between 2 to 4 log CFU ml^{-1} (Fig. 1). There was no statistical difference ($P = 0.43$) between the average *E. coli* load for large and small slaughterhouses (Table 1), suggesting that factors other than facility handling volume are important determinants of *E. coli* contamination. Nevertheless, meat samples from one large (C) and two small (F and G) facilities had relatively high loads of *E. coli* that exceeded the limit (<3 log CFU g^{-1}) recommended by one international food standards agency *(15)* compared with the other facilities (Table 1). Higher *E. coli* loads in these facilities might be related to site-specific sanitation and handling practices of carcasses or equipment *(1, 4),* but regardless of the reason these higher

TABLE 1. *Average log CFU of* E. coli *detected in goat meat samples (12 per house) from small and large slaughterhouses located at 10 different sites in Arusha, Tanzania*[a]

Slaughter facility site	*E. coli* load (log CFU ml^{-1})	95% confidence interval
Large facility ($n = 5$)	3.37 ± 0.12 A[b]	3.14–3.59
A	3.15 ± 0.26 A	2.36–3.76
B	3.29 ± 0.17 A	2.79–3.80
C	3.79 ± 0.12 A	3.44–4.15
D	3.41 ± 0.15 A	2.97–3.85
E	3.19 ± 0.15 A	2.75–3.64
Small facility ($n = 5$)	3.13 ± 0.25[b]	3.71–4.13
F	3.92 ± 0.07 B	2.65–3.62
G	3.51 ± 0.15 B	3.05–3.98
H	2.76 ± 0.07 A	2.54–2.98
I	2.69 ± 0.04 A	2.56–2.82
J	2.78 ± 0.14 A	2.34–3.21

[a] Values are means ± standard errors. Values followed by different letters designate significantly different groups by Tukey's honestly significant difference post hoc test at $P < 0.001$.

[b] Group mean for small or large facilities.

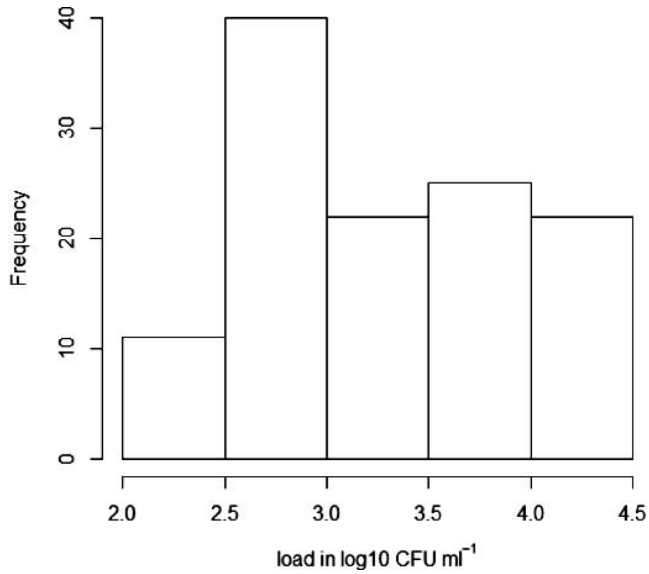

FIGURE 1. *Distribution of* E. coli *load from all goat meat samples collected from the Arusha district, Tanzania. Bars refer to the number of samples,* n = *120 positive samples.*

loads may represent a greater risk of transmission to consumers *(34)*. Within the large and small size groupings, there was a range of recoverable *E. coli* (Table 1). Inconsistent or different slaughter practices among goat meat handlers at different facilities possibly accounts for this discrepancy *(12)*.

The methodology used in the current study included collection of an initial meat sample (250 g) that was larger than conventional samples (100 g *(21)*), with the intention of collecting a more representative sample for testing. We then tested a 25-g subsample by preparing rinsate with 25 ml of sterile double-distilled water. Using this methodology we had no difficulty detecting *E. coli* (2 to 4 log CFU ml^{-1}) from goat meat samples from Arusha, but these numbers are relatively low compared with those from reports from low-income settings, where less intensive sampling yielded *E. coli* loads between 5.4 and 7.5 log CFU ml^{-1} *(6, 12)*. We observed that most premises frequently used hot water to clean equipment and work surfaces, and we surmise that the lower average loads observed in our study may be related to these practices.

Antibiotic susceptibility testing results (Table 2) for *E. coli* isolates (1,416 for large facilities and 1,320 for small facilities) indicated that meat samples from large facilities harbored a higher prevalence ($P = 0.026$) of antibiotic-resistant *E. coli* compared with small facilities. The highest prevalence of resistance was for ampicillin followed by amoxicillin. A Pearson's correlation coefficient indicated a

TABLE 2. *Average prevalence (%) of antibiotic-resistant* E. coli *in goat meat samples from five large-scale and five small-scale slaughterhouses in Arusha, Tanzania*[a]

Antibiotic	Large slaughterhouse ($n = 5$)	Small slaughterhouse ($n = 5$)	Overall mean
Ampicillin	30.1 ± 10.2 (11.9–38.1)	12.8 ± 1.1 (10.6–14.9)	21.5 ± 8.9 B
Amoxicillin	17.6 ± 5.4 (9.9–25.7)	4.5 ± 1.3 (1.9–7.0)	11.1 ± 6.5 AB
Streptomycin	5.2 ± 0.9 (3.5–6.9)	3.9 ± 1.1 (1.8–6.1)	4.7 ± 0.6 A
Sulfamethoxazole	7.1 ± 2.2 (2.8–11.5)	5.1 ± 1.3 (2.5–7.7)	5.7 ± 0.7 A
Tetracycline	4.6 ± 0.7 (3.3–5.9)	10.6 ± 2.3 (6.0–15.2)	7.6 ± 0.7 A
Trimethoprim	5.1 ± 1.7 (1.7–8.5)	3.9 ± 1.7 (0.6–7.2)	4.6 ± 0.6 A
Overall mean	11.6 ± 4.2 A	6.8 ± 1.6 B	
F statistic			
Antibiotic	6.44***[b]		
Slaughter scale	5.27*		
Antibiotic × slaughter scale	2.88*		

[a] Comparisons are based on a two-way analysis of variance to compute the *F* statistic and Tukey's honestly significant difference post hoc test. Values are means ± standard errors, with 95% confidence intervals in parentheses. Values followed by different letter(s) in the same column or row designate significantly different groups by Tukey's honestly significant difference post hoc at $P < 0.001$. All the *E. coli* isolates, regardless of source, were susceptible to ceftazidime and cefotaxime. Chloramphenicol and ciprofloxacin were not analyzed because of insufficient data.

[b] * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 3. *Average prevalence (%) of antibiotic-resistant* E. coli *in goat meat samples from different sites within large slaughterhouses in Arusha, Tanzania*[a]

	Antibiotic[b]:						
Large facility ($n = 5$)	Amp	Amx	Str	Sul	Tet	Tri	Overall mean
A	10.2 ± 5.3	4.9 ± 2.3	2.3 ± 1.2	1.9 ± 1.6	4.9 ± 2.4	2.7 ± 1.0	4.4 ± 1.2 A
B	12.1 ± 3.9	8.7 ± 4.1	6.9 ± 2.9	7.3 ± 2.2	4.2 ± 2.4	5.2 ± 1.6	7.2 ± 1.0 A
C	49.7 ± 10.7	35.4 ± 11	6.6 ± 5.6	12.8 ± 6.0	6.6 ± 2.9	11.8 ± 6.7	20.5 ± 7.3 C
D	58.7 ± 9.7	22.2 ± 6.8	4.2 ± 2.6	11.1 ± 4.9	2.4 ± 1.2	3.1 ± 2.1	16.9 ± 8.9 BC
E	20.5 ± 8.3	15.6 ± 8.3	5.9 ± 4.1	2.4 ± 1.1	4.9 ± 3.2	2.8 ± 1.7	8.7 ± 3.1 AB
Overall mean	30.1 ± 10.2 C	17.6 ± 5.4 B	5.2 ± 0.9 A	7.1 ± 2.2 A	4.6 ± 0.7 A	5.1 ± 1.7 A	
F statistic							
Antibiotic	19.72***[c]						
Site	10.14***						
Antibiotic × site	3.11***						

[a] Values are means ± standard errors. Comparisons are based on a two-way analysis of variance to compute the *F* statistic and Tukey's honestly significant difference post hoc test. Values followed by different letter(s) in the same column or row designate significantly different groups by Tukey's honestly significant difference post hoc test at $P < 0.001$.
[b] Amp, ampicillin; Amx, amoxicillin; Str, streptomycin; Sul, sulfamethoxazole; Tet, tetracycline; Tri, trimethoprim.
[c] * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

positive correlation between ampicillin and amoxicillin resistance among isolates in large and small facilities ($r = 0.60$ and $r = 0.67$, respectively; $P < 0.001$). This is consistent with the presence of both independent resistance traits and cross-protective resistance traits in the *E. coli* population. Moreover, the statistical interaction ($P < 0.05$) between slaughterhouse size and antibiotics (plot not shown) indicated that *E. coli* resistance to ampicillin and tetracycline was higher in large ($P < 0.001$) and small ($P = 0.018$) slaughterhouses, respectively (Table 2).

The higher prevalence of *E. coli* resistance to ampicillin, amoxicillin, and tetracycline might be related to the common use of antibiotics that belong to the penicillin and tetracycline drug classes. Tanzanian farmers often treat livestock without a veterinary prescription or veterinary supervision *(20)*. Similar observations were reported in the neighboring country Kenya *(22, 29)*. Despite several reports about antibiotic use practices in Tanzanian livestock production *(17),* no studies have been done to directly assess the impact of these practices. Nevertheless, it is important to note that even in the absence of antibiotic use, resistant populations can persist in livestock populations, on environmental surfaces, and in water and can even come from people *(2, 7)*.

Among the large facilities, the overall prevalence of antibiotic-resistant *E. coli* from meat samples was significantly higher ($P < 0.001$) at facilities C and D compared with facilities A, B, and E (Table 3). There was a statistical interaction ($P < 0.001$) between sampling sites and antibiotic type for isolates analyzed from large slaughter-

TABLE 4. *Average prevalence (%) of antibiotic-resistant* E. coli *in goat meat samples from different sites within small slaughterhouses in Arusha, Tanzania*[a]

	Antibiotic[b]:						
Small facility ($n = 5$)	Amp	Amx	Str	Sul	Tet	Tri	Overall mean
F	15.0 ± 7.1	2.9 ± 1.9	5.4 ± 2.7	4.6 ± 2.9	10.8 ± 6.4	2.5 ± 1.7	7.8 ± 2.1 A
G	13.5 ± 4.1	4.5 ± 2.5	6.6 ± 1.8	4.5 ± 2.3	3.1 ± 1.5	3.5 ± 1.9	5.3 ± 1.5 A
H	14.6 ± 5.8	2.5 ± 1.4	—[c]	6.3 ± 3.5	8.3 ± 5.1	—	10.1 ± 2.9 A
I	11.7 ± 7.5	9.5 ± 8.3	4.2 ± 3.2	9.1 ± 8.3	15.9 ± 8.7	10.2 ± 7.7	5.9 ± 1.1 A
J	9.0 ± 3.0	3.1 ± 2.2	3.5 ± 2.4	1.0 ± 0.5	14.9 ± 5.6	3.5 ± 2.3	5.9 ± 1.3 A
Overall mean	12.8 ± 1.1 C	4.5 ± 1.3 ABC	3.9 ± 1.1 A	5.1 ± 1.3 ACD	10.6 ± 2.3 AC	3.9 ± 1.7 A	
F statistic							
Antibiotic	3.59**[d]						
Site	1.09[NS]						
Antibiotic × site	0.49[NS]						

[a] Values are means ± standard errors. Comparisons are based on a two-way analysis of variance to compute the *F* statistic and Tukey's honestly significant difference post hoc test. Values followed by different letter(s) in the same column or row designate significantly different groups by Tukey's honestly significant difference post hoc test at $P < 0.05$.
[b] Amp, ampicillin; Amx, amoxicillin; Str, streptomycin; Sul, sulfamethoxazole; Tet, tetracycline, Tri, trimethoprim.
[c] —, antibiotic not detected.
[d] * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, nonsignificant ($P > 0.05$).

TABLE 5. *Prevalence (%) of various antimicrobial-resistant (AMR) phenotypes and susceptible isolates among* E. coli *in goat meat samples collected from five large and five small slaughterhouses in Arusha district, Tanzania*

AMR phenotype[a]	Large slaughter facilities ($n = 5$)	Small slaughter facilities ($n = 5$)
Amp	10.7	5.9
Amx	1.6	0.4
Chl	—[b]	0.1
Str	1.9	1.7
Sul	0.7	0.6
Tet	2.1	6.4
Tri	0.9	0.1
AmpAmx	8.7	1.1
AmpChl	0.7	—
AmpStr	0.4	0.4
AmpSul	1.1	0.8
AmpTet	0.6	1.3
AmpTri	0.7	—
AmxChl	0.1	—
AmxSul	0.1	—
AmxTet	0.1	0.1
AmxTri	0.1	0.1
ChlTet	—	0.1
StrSul	0.1	—
StrTri	0.1	1.5
SulTet	—	0.3
SulTri	0.1	0.1
TetTri	0.1	—
AmpAmxTet	0.1	—
AmpAmxChl	0.4	—
AmpAmxStr	0.4	—
AmpAmxSul	2.1	0.2
AmpAmxTet	0.6	0.1
AmpAmxTri	0.2	—
AmpChlTet	0.1	—
AmpStrSul	0.1	—
AmpSulTet	0.1	0.3
AmpSulTri	0.1	0.1
AmpTetTri	2.8	—
AmxCipTet	0.1	—
AmxStrSul	0.1	
AmxStrTri	0.1	—
StrTetTri	—	0.1
AmpAmxChlTri	0.1	—
AmpAmxStrTri	0.1	—
AmpAmxStrSul	0.6	0.1
AmpAmxSulTet	0.1	0.2
AmpAmxSulTri	0.5	0.5
AmpAmxTetTri	0.1	—
AmpChlTetTri	0.2	—
StrSulTetTri	0.1	—
AmpAmxStrSulTri	1.2	0.1
AmpAmxSulTetTri	—	0.1
AmpAmxCipSulTetTri	—	0.4
AmpAmxCipSulTetTri	—	1.3
AmpAmxCipChlSulTetTri	—	0.1
AmpAmxCipChlStrSulTri	0.1	—
Total AMR phenotypes	41.3	24.6
Susceptible isolates	58.7	75.4

[a] Amp, ampicillin; Amx, amoxicillin; Cip, ciprofloxacin; Chl, chloramphenicol; Str, streptomycin; Sul, sulfamethoxazole; Tet, tetracycline; Tri, trimethoprim.

[b] —, antibiotic not detected.

houses, and this interaction was attributable to changes in the rank order of resistance for site C relative to sites A and B and for site D relative to sites A, B, and E. Site D had a significantly higher prevalence ($P < 0.001$) of ampicillin resistance compared with site C. Generally, sites C and D yielded more resistant *E. coli* compared with other sites. These two sites are located in a concentrated commercial area of Arusha known as *Muromboo* that is famous for vending roasted goat meat. Qualitatively, this area has the highest density of live goat auctions and slaughter operations that might contribute to more transmission of antibiotic-resistant bacteria with meat. For example, others have reported a positive relationship between the numbers of animals that are processed and recovery of bacterial contamination from the meat products *(32, 37)*.

Within small slaughterhouses there was no significant difference ($P > 0.05$) among sites (Table 4). Overall the rank order for prevalence of antibiotic-resistant *E. coli* resistance was comparable to that of large facilities, with isolates showing more resistance ($P < 0.05$) to ampicillin than to other antibiotics. For this comparison there was no statistical interaction ($P > 0.05$) between sample sites and antibiotic type.

Generally, in most farming communities, the environment is shared between animals and people; therefore, both can share resistant bacteria with additional transmission through handling of meat products *(9)*. Management practices might contribute to the size effect as well. For example, experience from veterinary officers in this region indicates that large facilities have clients with larger herds and more animal stock, and they have greater potential to use more antibiotics (presumably because of increased risk of disease spread) to treat animals during or before being transported to a slaughterhouse. These practices would probably selectively enrich resistant populations of bacteria, as was observed at the larger facilities in this study. Alternatively, the act of transporting animals may be compatible with more transmission of antibiotic-resistant bacteria derived from other sources such as environmental surfaces, water, people, and other animals. Moreover, during antemortem examination in large slaughterhouses, animals are crowded at one location in a high density, thereby increasing the probability of transmission of bacteria *(27)*. Regardless, our findings are consistent with the possibility that larger slaughter operations might be an important foci for transmitting antibiotic-resistant bacteria in meat. With the demand for meat driving the growth of larger facilities, this demand could become a problem as economies develop, at least until industry standardization is adopted.

The limited prevalence of chloramphenicol-resistant *E. coli* (1.5% in large facilities and 0.2% in small facilities) observed in this study is encouraging because it suggests that chloramphenicol is not used routinely in Tanzanian food animals. This is important because exposure to chloramphenicol can cause a dose-independent incidence of aplastic anemia in people and subsequent increased risk of B-cell lymphoma *(40)*. Unfortunately, chloramphenicol is used to treat food animals in other countries *(30)*, and the use of a veterinary analog, florfenicol, can select for cross-resistance if resistance is conferred by a florfenicol efflux pump *(11)*.

Interestingly, only 5.6% of the 2,736 isolates were resistant to three or more antibiotic classes. Of these isolates, 108 (70.6%) were recovered from large facilities and 45 (29.4%) from small facilities. Two isolates were resistant to up to seven of the tested antibiotics. Overall, there were 53 different resistance phenotypes (including susceptible strains) of which only 11 were found at prevalence level >1% (Table 5). This limited prevalence of multidrug resistance is comparable to what Gousia et al. *(18)* reported for goats in Greece, but it is considerably lower than the 88% that has been reported for *Salmonella* isolated from water sources in northern Tanzania *(24)*. The relatively low prevalence of multidrug-resistant *E. coli* could be indicative of relatively low exposure to antibiotics, on average, for the farms that use these slaughter facilities. This low prevalence may change, however, as larger herds are developed to meet growing consumer demand.

Findings from this study show that the total load of fecal *E. coli* contamination of goat meat from slaughter facilities in Arusha is within the range of recommended limits set by one international food standards agency *(15)*. Carriage of commensal AMR *E. coli,* including some multidrug-resistant strains in goat meat from this area, could be a concern to consumers if undercooked meat is consumed. Management practices in large slaughter operations might play an important role in selecting for larger populations of resistant bacteria and contributing to a higher burden of antibiotic-resistant bacteria on meat products. More effective hygiene measures during slaughter and posthandling of meat are recommended to limit the magnitude of meat contamination.

ACKNOWLEDGMENTS

The authors acknowledge the Paul G. Allen School for Global Animal Health, the Nelson Mandela African Institution of Science and Technology, and the National Science Foundation (DEB1216040) for financial support for part of this study.

REFERENCES

1. Adetunde, L. A., R. L. K. Glover, A. W. O. Oliver, and T. Samuel. 2011. Source and distribution of microbial contamination on beef and chevon in Navrongo, Kassena Nankana District of Upper East Region in Ghana. *Anim. Prod.* 1:21–28.
2. Allen, H. K., J. Donato, H. H. Wang, K. A. Cloud-Hansen, J. Davies, and J. Handelsman. 2010. Call of the wild: antibiotic resistance genes in natural environments. *Nat. Rev. Microbiol.* 8:251–259.
3. Bailey, J. K., J. L. Pinyon, S. Anantham, and R. M. Hall. 2010. Commensal *Escherichia coli* of healthy humans: a reservoir for antibiotic-resistance determinants. *J. Med. Microbiol.* 59:1331–1339.
4. Bhandare, S. G., A. T. Sherikar, A. M. Paturkar, V. S. Waskar, and R. J. Zende. 2007. A comparison of microbial contamination on sheep/goat carcasses in a modern Indian abattoir and traditional meat shops. *Food Control* 18:854–858.
5. Boschi-Pinto, C. 2008. Estimating child mortality due to diarrhoea in developing countries. *Bull. W.H.O.* 86:710–717.
6. Bradeeba, K., and P. K. Sivakumaar. 2012. Assessment of microbiological quality of beef, mutton and pork and its environment in retail shops in Chidambaram, Tamil Nadu. *Int. J. Plant Anim. Emviron. Sci.* 3:91–97.
7. Capita, R., and C. Alonso-Calleja. 2013. Antibiotic-resistant bacteria: a challenge for the food industry. *Crit. Rev. Food Sci. Nutr.* 53:11–48.
8. Clinical and Laboratory Standards Institute. 2007. Performance standards for antimicrobial susceptibility testing; seventeenth informational supplement. Available at: http://microbiolab-bg.com/wp-content/uploads/2015/05/CLSI.pdf. Accessed 1 December 2015.
9. Davies, J., and D. Davies. 2010. Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev.* 74:417–433.
10. Dhama, K., S. Rajagunalan, S. Chakraborty, A. K. Verma, A. Kumar, R. Tiwari, and S. Kapoor. 2013. Food-borne pathogens of animal origin-diagnosis, prevention, control and their zoonotic significance: a review. *Pak. J. Biol. Sci.* 16:1076–1085.
11. Du, X., C. Xia, J. Shen, B. Wu, and Z. Shen. 2004. Characterization of florfenicol resistance among calf pathogenic *Escherichia coli*. *FEMS Microbiol. Lett.* 236:183–189.
12. Eze, V. C., and N. Ivuoma. 2012. Evaluation of microbial quality of fresh goat meat sold in Umuahia market, Abia state, Nigeria. *Pak. J. Nutr.* 11:782–786.
13. Farrokh, C., K. Jordan, F. Auvray, K. Glass, H. Oppegaard, S. Raynaud, D. Thevenot, R. Condron, K. De Reu, A. Govaris, K. Heggum, M. Heyndrickx, J. Hummerjohann, D. Lindsay, S. Miszczycha, S. Moussiegt, K. Verstraete, and O. Cerf. 2013. Review of Shiga-toxin-producing *Escherichia coli* (STEC) and their significance in dairy production. *Int. J. Food Microbiol.* 162:190–212.
14. Food and Agriculture Organization of the United Nations, World Health Organization. 2014. Risk manager's guide to the statistical aspects of microbiological criteria related to foods. Available at: ftp://ftp.fao.org/codex/meetings/CCFH/CCFH46/FAO%20MC%20draft%20140814a.pdf. Accessed 27 November 2015.
15. Food Standards Agency. 2005. General guidance for food business operators on microbiological criteria for foodstuffs. Available at: http//www.foodlaw.rdg.ac.uk/pdf/uk-06001-micro-criteria.pdf. Accessed 3 September 2015.
16. Frederick, A., T. G. Ayum, A. A. Gifty, and A. Samuel. 2010. Microbial quality of chevon and mutton sold in Tamale Metropolis of northern Ghana. *J. Appl. Sci. Environ. Manag.* 14:53–55.
17. Global Antibiotic Resistance Partnership-Tanzania Working Group. 2015. Situation analysis: antibiotic use and resistance in Tanzania. Available at: http://www.cddep.org/publications/garp_tanzania_situation_analysis. Accessed 3 December 2015.
18. Gousia, P., V. Economou, H. Sakkas, S. Leveidiotou, and C. Papadopoulou. 2011. Antimicrobial resistance of major foodborne pathogens from major meat products. *Foodborne Pathog. Dis.* 8:27–38.
19. Grace, D. 2015. Review of evidence on antimicrobial resistance and animal agriculture in developing countries. The National Archives, Surrey, UK.
20. Katakweba, A. A. S., M. M. A. Mtambo, and J. E. Olsen. 2012. Awareness of human health risks associated with the use of antibiotics among livestock keepers and factors that contribute to selection of antibiotic resistance bacteria within livestock in Tanzania. *Livest. Res. Rural Dev.* 24.
21. Kiiyukia, C. 2003. Laboratory manual of food microbiology for Ethiopian Health and Nutrition Research Institute. United Nations Development Organization Project Report YA/ETH/03/436/11-52. Available at: http://www.unido.org/fileadmin/media/documents/pdf/Agro/MacroLab.pdf. Accessed 12 December 2015.
22. Kikuvi, G. M., E. S. I. Ole-Mapenay, M. Mitema, and J. Ombui. 2013. Antimicrobial resistance in *Escherichia coli* isolates from faeces and carcass samples of slaughtered cattle, swine and chickens in Kenya. *Isr. J. Vet. Med.* 61:82–88.
23. Kuchenmüller, T., S. Hird, C. Stein, P. Kramarz, A. Nanda, and A. H. Havelaar. 2009. Estimating the global burden of foodborne diseases—a collaborative effort. *Euro Surveill.* 14(18):pii=19195.
24. Lyimo, B., J. Buza, W. Smith, M. Subbiah, and D. R. Call. 2016. Surface waters in northern Tanzania harbor fecal coliform and antibiotic resistant *Salmonella* spp. capable of horizontal gene transfer. *Afr. J. Microbiol. Res.* 10:348–356.
25. Mensa, P., L. Mwamakamba, C. Mohamed, and D. Nsue-Milang. 2012. Public health and food safety in the WHO African region. *Afr. J. Food Agric. Nutr. Dev.* 12:1–19.
26. Nawaz, M., A. A. Khan, S. Khan, K. Sung, K. Kerdahi, and R. Steele. 2009. Molecular characterization of tetracycline-resistant genes and

integrons from avirulent strains of *Escherichia coli* isolated from catfish. *Foodborne Pathog. Dis.* 6:553–559.
27. Ndubueze, A., I. Nwakno, and S. Olu. 2015. Antibiotic resistance in *Escherichia coli* isolate from healthy food animals in Ngor Okpala, Imo State, Nigeria. *J. Agric. Vet. Med.* 8:55–59.
28. Netherlands Development Organisation. 2008. Red meat for local and export markets sub sector analysis Tanzania. SNV Netherlands Development Organisation, The Hague, The Netherlands.
29. Njoroge, S., A. W. T. Muigai, P. N. Njiruh, and S. Kariuki. 2013. Molecular characterization and antimicrobial resistance patterns of *Escherichia coli* isolates from goats slaughtered in parts of Kenya. *East Afr. Med. J.* 1:1–11.
30. Nsofor, C., A. I. O. Olatoye, E. A. Amosun, C. U. Iroegbu, M. A. Davis, L. H. Orfe, and D. R. Call. 2013. *Escherichia coli* from Nigeria exhibit a high prevalence of antibiotic resistance where reliance on antibiotics in poultry production is a potential contributing factor. *Afr. J. Microbiol. Res.* 7:4646–4654.
31. Odu, N. N., and I. I. Okonko. 2013. Microbiological analysis of kilishi sold in Port Harcourt, Nigeria. *N. Y. Sci. J.* 6:37–43.
32. Odwar, J. A., G. Kikuvi, J. N. Kariuki, and S. Kariuki. 2014. A cross-sectional study on the microbiological quality and safety of raw chicken meats sold in Nairobi, Kenya. *BMC Res. Notes* 7:627.
33. Sabarinath, A., K. P. Tiwari, C. Deallie, G. Belot, G. Vanpee, V. Matthew, R. Sharma, and H. Hariharan. 2011. Antimicrobial resistance and phylogenetic groups of commensal Escherichia coli isolates from healthy pigs in Grenada. *Webmed Cent. Vet. Med.* 2:1–10.
34. Stoica, M., S. Stoean, and P. Alexe. 2014. Overview of biological hazards associated with the consumption of the meat products. *J. Agroaliment. Process. Technol.* 20:192–197.
35. Subbiah, M., E. M. Top, D. H. Shah, and D. R. Call. 2011. Selection pressure required for long-term persistence of blaCMY-2-positive IncA/C plasmids. *Appl. Environ. Microbiol.* 77:4486–4493.
36. Sutton, S. 2011. Accuracy of plate counts. *J. Valid. Technol.* 17:42–46.
37. Ukut, I. O. E., I. O. Okonko, I. S. Ikpoh, A. O. Nkang, A. O. Udeze, T. A. Babalola, O. K. Mejeha, and E. A. Fajobi. 2010. Assessment of bacteriological quality of fresh meats sold in Calabar Metropolis, Nigeria. *Electron. J. Environ. Agric. Food Chem.* 9:89–100.
38. United Nations Industrial Development Organization. 2012. Tanzania's red meat value chain: a diagnostic. African Agribusiness and Agro-Industries Development Initiative. United Nations Industrial Development Organization, Vienna.
39. Vernet, G., C. Mary, D. M. Altmann, O. Doumbo, S. Morpeth, Z. A. Bhutta, and K. P. Klugman. 2014. Surveillance for antimicrobial drug resistance in under-resourced countries. *Emerg. Infect. Dis.* 20:434–441.
40. Yuan, Z. R., and Y. Shi. 2008. Chloramphenicol induces abnormal differentiation and inhibits apoptosis in activated T cells. *Cancer Res.* 68:4875–4881.