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Isolation and characterization of antimicrobial-resistant *Escherichia coli* from retail meats from roadside butcherries in Uganda

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ABSTRACT

Retail meats are one of the main routes for spreading antimicrobial-resistant bacteria from livestock to humans through the food chain. In African countries, retail meats are often sold at roadside butcheries without chilling or refrigeration. Retail meats in those butcheries are suspected to be contaminated by antimicrobial-resistant bacteria, but it was not clear. In the present study, we tested for the presence of antimicrobial-resistant *Escherichia coli* from retail meats (n = 64) from roadside butcheries in Kampala, Uganda. The meat surfaces were swabbed and inoculated on PetriFilm SEC agar to isolate *E. coli*. We successfully isolated *E. coli* from 90.6% of these retail meat samples. We identified the phylogenetic type, antimicrobial susceptibility, and antimicrobial resistance genes prevalence between retail meat isolates (n = 89). Phylogenetic type B1 was identified from 70.8% of the retail meat isolates, suggesting that the isolates originated primarily from fecal contamination during meat processing. Tetracycline-resistant isolates with *tetA* and/or *tetB* gene(s) were the most frequently detected (28.1%), followed by ampicillin-resistance genes with *bla*_{TEM} (15.7%,) and sulfamethoxazole-trimethoprim resistance genes with *sul2* (15.7%). No extended-spectrum beta-lactamase-producing isolates were detected. A conjugation assay showed that resistance to ampicillin, tetracycline, and sulfamethoxazole-trimethoprim could be simultaneously transferred to recipients. These findings suggest that antimicrobial-resistant *E. coli* can easily be transferred from farms to tables from retail meats obtained from roadside butcheries.

Introduction

The spread of antimicrobial-resistant bacteria (ARB) is a global concern in both human and veterinary medicine (World Health Organization, 2015). ARB in food-producing animals presents a risk of disseminating ARB from animals to humans through the food chain (Economou and Gousia, 2015; Founou et al., 2016). As fecal bacteria in livestock can easily be transferred to retail meats in a slaughterhouse during meat processing, retail meats are one of the most important routes for spreading ARB from livestock to humans, or “farm-to-table” (Aslam et al., 2003; Schroeder et al., 2004). Many researchers have reported the detection and prevalence of ARB in retail meat samples (Eyi and Arslan, 2012; Johnson et al., 2009; Martínez-Vázquez et al., 2018; Zhao et al., 2012). Some studies have reported isolating clinically important ARB, such as extended-spectrum beta-lactamase (ESBL) producing bacteria, from retail meat samples (Ye et al., 2018). However, most of these studies were conducted in developed countries with improved food chains and appropriate cooling systems, and ARB studies of retail meats from developing countries remain limited (Messele et al., 2017).

Retail meats in developed countries are usually distributed in cold chains (Nastasijević et al., 2017). In general, beef carcasses are chilled immediately after slaughtering in chilled rooms for 24 - 96 hours. They are kept in chilling rooms and then cut into primary cuts for distribution. Some studies reported that the numbers of lactic acid bacteria and *Pseudomonas* on the chilled meat surfaces were increased, while the number of *Enterobacteriaceae* (*Salmonella* Enteritidis) was slightly decreased (Chenoll et al., 2007; Ercolini et al., 2006; Sabike et al., 2015). Conversely, roadside butcheries in developing countries purchase their retail meats from slaughterhouses in the morning and sell them on the same day without chilling because these butcheries are rarely equipped with a refrigerator or freezer (Fig 1). In these countries, retail meats are commonly transported directly from the slaughterhouses to retailers on bicycles or light trucks. Thus, we considered that retail meats from roadside butcheries clearly reflect the prevalence of ARB among livestock in

Uganda. To demonstrate the possibility of spreading ARB via retail meats from roadside butcheries, here we report the prevalence and the characteristics (phylogenetic type, antimicrobial susceptibility, and resistance genes) of antimicrobial-resistant *E. coli*, a major fecal indicator bacterium, isolated from retail meats in Uganda (Johnson et al., 2009; Schroeder et al., 2004).

Materials and Methods

Between February and October 2018, 64 retail meat samples (60 beef ribs and 4 goat ribs) were purchased at roadside butcheries (n = 64) in Kampala, Uganda (Fig. 1). One piece (quarter or half kilogram) was bought at each of butcheries. Retail meats in the roadside butcheries were slaughtered on the same day at slaughterhouses in Kampala. We couldn't trace which slaughterhouse had processed the meat we bought. The samples were transported to the laboratory at ambient temperature and processed within 3 hours after collection. The meat sample surfaces (approximately 30 cm × 10 cm) were swabbed completely with sterilized cotton swabs moistened by sterilized 0.85% saline solution (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) and suspended in 1 mL of saline solution. After serial dilution, 1 mL of the solution was inoculated on a PetriFilm SEC plate (3M Company, MN, USA) and cultured at 37°C overnight. Up to 2 representative blue colonies (presence of β-galactosidase) were subcultured on ES Colimark agar media (Eiken Chemical Co., Ltd., Tokyo, Japan). Single blue colonies (presence of β-glucuronidase) were defined as *E. coli* isolates and were checked via *E. coli*-specific PCR (Wang et al., 1996).

PCR-based phylogenetic typing of *E. coli* was performed to classify the isolates into seven groups and subgroups: A₀, A₁, B₁, B₂, B₂₃, D₁, and D₂ (Escobar-Páramo et al., 2004). *E. coli* belonging to groups A₀, A₁, and B₁ are usually commensal and nonpathogenic strains. Groups B₂ and B₂₃ include extra-intestinal pathogenic strains related to urinary tract infections, and groups D₁ and D₂ include intra-intestinal pathogenic strains.

104 The minimum inhibitory concentrations (MICs) for 12 antimicrobial agents (ampicillin
105 [AMP], cefazolin [CFZ], cefotaxime [CTX], gentamicin [GEN], kanamycin [KAN], tetracycline
106 [TET], minocycline [MIN], nalidixic acid [NAL], ciprofloxacin [CIP], colistin [CST],
107 chloramphenicol [CHL], and sulfamethoxazole-trimethoprim [SXT]) were tested via broth
108 microdilution method using frozen plates (Eiken Chemical Co., Ltd.) according to the manufacturer's
109 instructions. The breakpoint MICs of these antimicrobial agents were determined according to the
110 Clinical and Laboratory Standards Institute guideline M100 ED29 (Clinical and Laboratory
111 Standards Institute, 2019). Breakpoint to CST was defined as ≥ 4 mg/L according to the European
112 Committee on Antimicrobial Susceptibility Testing guideline (European Committee on Antimicrobial
113 Susceptibility Testing, 2019).

114 To prevent analysis of duplicated strains, *E. coli* isolates from the same meat samples with
115 the same phylogenetic type and antimicrobial susceptibility pattern were omitted from further
116 analysis. Colony-direct PCR was performed using Quick Taq HS DyeMix (TOYOBO Co., Ltd.,
117 Osaka, Japan) to detect the following antimicrobial resistance genes: *bla*_{TEM}, *bla*_{SHV}, and *bla*_{OXA} for
118 AMP-resistant strains; *tetA*, *tetB*, *tetC*, *tetD*, *tetE*, and *tetG* for TET-resistant strains; and *sul1*, *sul2*,
119 and *sul3* for SXT-resistant strains (Colom et al., 2003; Phuong Hoa et al., 2008; Vignaroli et al.,
120 2012).

121 We tested the transferability of antimicrobial resistance genes from the retail meat isolates to
122 other *E. coli* via conjugation assay by broth mating method (Potron et al., 2011).
123 AMP-TET-SXT-resistant isolates were used as donors, and sodium azide-resistant *E. coli* J53 was
124 used as the recipient. The donor and recipient were mixed at a 1:1 ratio (final concentration of both
125 10^7 colony-forming units [CFU]) in phosphate-buffered saline and incubated at 37°C for 18 hours.
126 We focused on the transfer of AMP-resistance because (1) AMP is reported to be one of the most
127 commonly-used first-line antibiotics in East Africa; and (2) usage of AMP is recommended by the
128 WHO Integrated Management of Childhood Illness for treatment of bacterial infections in infants

129 (Ampaire et al., 2016). After mating, the solution was inoculated on LB agar (Nakarai Tesque Inc.,
130 Kyoto, Japan) containing 100 mg/L of sodium azide with or without 50 mg/L of AMP. After
131 overnight cultivation at 37°C, the number of colonies on selective plates was counted manually. Then
132 we calculated the CFU of transconjugants and recipients in the mating solution. Transfer frequencies
133 were determined as the number of transconjugants per recipient. The transfer of antimicrobial
134 resistance gene(s) was confirmed via PCR using representative recipient colonies.

135

136

137 Results

138 We isolated 103 *E. coli* isolates from 58 retail meat samples (58/64; 90.6%). Among them,
139 14 had the same phylogenetic type and antimicrobial susceptibility pattern and were thus suspected
140 to be duplicated clones and omitted from further analysis. Therefore, we used 89 *E. coli* isolates in
141 this study. The phylogenetic patterns of retail meat isolates were as follows: A₀, 4.5% (4/89); A₁,
142 7.9% (7/89); B₁, 70.8% (63/89); B₂, 2.2% (2/89); B₂₃, undetected; D₁, 13.5% (12/89); and D₂, 1.1%
143 (1/89).

144 Antimicrobial susceptibility testing showed that TET-resistant isolates were the most
145 frequently detected (28.1%, 25/89), followed by AMP-resistant isolates (15.7%, 14/89) and
146 SXT-resistant isolates (15.7%, 14/89; Fig. 2). Only a few isolates showed resistance to GEN (3.4%,
147 3/89), NAL (2.2%, 2/89), CIP (1.1%, 1/89), or CHL (1.1%, 1/89). Resistance to cephalosporins (CFZ
148 and CTX), KAN, MIN, or CST was not detected. As for multidrug resistance, 10 isolates (11.2%)
149 showed simultaneous AMP-TET-SXT resistance (Table 1).

150 All AMP-resistant retail meat isolates (14/14) harbored the *bla*_{TEM} gene. TET-resistant retail
151 meat isolates harbored several *tet* genes: *tetA*, 68.0% (17/25); *tetB*, 32.0% (8/25); *tetE*, 4.0% (1/25);
152 and *tetG*, 4.0% (1/25). Two isolates harbored both *tetA* and *tetB*. The most prevalent SXT-resistance
153 gene was *sul2* (14/14, 100%), and 7 isolates (50.0%) simultaneously harbored *sul2* and *sul1*. *sul3*

was not detected.

We tested the transferability of AMP resistance using multidrug (AMP-TET-SXT) resistant isolates (n = 10). Among them, 6 isolates successfully transferred their AMP resistance to *E. coli* J53. The transfer frequencies of those isolates were 10^{-3} to 10^{-4} (Table 1). PCR for antimicrobial-resistant genes showed that transconjugants derived from those 6 multidrug-resistant donors harbored the AMP-resistant gene, *bla*_{TEM}, and the TET- and SXT-resistant genes, *tetA*, *tetB*, *sul1*, and/or *sul2*. On the other hand, AMP-resistance determinants in 4 isolates didn't transfer to *E. coli* J53. The transfer frequencies of those isolates were lower than 10^{-7} to 10^{-8} (Table 1).

Discussion

Enteric bacterial pathogens such as *E. coli* on retail meats are mainly originated from fecal contamination of animal intestinal contents during meat processing (Brashears and Chaves, 2017). Among the retail meat samples in this study, 90.6% (58/64) were positive for *E. coli*. This frequency was relatively high compared with those of similar studies. *E. coli* prevalence in retail beef was reported to be 25.0% (14/56) in northwest Turkey, 49.2% (29/59) in Mexico, 68.9% (2,061/2,991) in the US, and 75.0% (27/36) in Thailand (Eyi and Arslan, 2012; Martínez-Vázquez et al., 2018; Zhao et al., 2012). The information on bacterial contamination in retail meats in East Africa is limited, but Azege *et al.* reported that 70.0% (21/30) of retail meats in the butcher shops in Ethiopia are contaminated by *Salmonella* (Azage and Kibret, 2017). We hypothesized that the widespread detection of *E. coli* in Ugandan beef was due to the distribution network of the retail meats because the meats were slaughtered and directly transported from slaughterhouses to roadside butcherries on the same day. In addition, cross-contamination of *E. coli* between meats could have occurred because little attention appeared to be paid to hygiene control at the roadside butcherries (Fig. 1) or during transportation, resulting in a high prevalence of *E. coli* on the meat surfaces.

179 PCR-based phylogenetic typing showed that group B1 was the most frequently detected
180 phylotype from retail meats (70.8%), followed by groups D₁ (13.5%) and A₀ (7.9%). *E. coli* groups
181 A₀, A₁, and B1 are commensal strains that include non- or low-pathogenic *E. coli* in the animal
182 digestive tract (Carlos et al., 2010). The high prevalence of *E. coli* group B1 from retail meats is
183 consistent with a previous report that group B1 was mainly isolated from the feces of herbivorous
184 animals, such as cows, and in retail meats (Carlos et al., 2010; Johnson et al., 2009). In addition, our
185 previous study reported that phylotype B1 was also prevalent among fecal *E. coli* isolates from
186 Ugandan livestock (Okubo et al., 2019). In agreement with those reports, our results suggest that *E.*
187 *coli* in the livestock digestive tract can be distributed from farms to consumers directly via retail
188 meats, especially under the conditions of a poor temperature-controlled supply chain.

189 Antimicrobial susceptibility testing showed that TET resistance was most frequently
190 detected in *E. coli* isolates from retail meats in Uganda. The main TET-resistance determinant was
191 the *tetA* gene, followed by the *tetB* gene. Our previous study revealed that TET resistance is common
192 among *E. coli* from Ugandan livestock, and most of these bacteria harbored *tetA* and/or *tetB* gene(s)
193 (Okubo et al., 2019). Another global study reported that human-derived TET-resistant atypical
194 enteropathogenic *E. coli* was significantly more common among isolates from East Africa (60%) and
195 West Africa (72%) compared with those in Asia (41%), suggesting that TET resistance is common
196 among *E. coli* in African countries regardless of the host (Ingle et al., 2018). Resistance to AMP and
197 SXT was also detected, and the major resistance determinants were *bla*_{TEM} and *sul2*, respectively.
198 Those genes were frequently detected from *Enterobacteriaceae* in Ugandan livestock samples in
199 previous studies (Odoch et al., 2018; Okubo et al., 2019), suggesting that the prevalence of TET-,
200 AMP-, or SXT-resistant *E. coli* and its resistance genes were common features among
201 livestock-derived bacteria in Uganda. AMP, TET, SXT and their derivatives are common antibiotics
202 in Ugandan livestock farms (Okubo et al., 2019). This indicates that ARB on retail meats are
203 coincident with what antibiotics the farmers used in livestock farms.

204 As for multidrug-resistance, 10 isolates showed AMP-TET-SXT resistance. Among them, 2
205 isolates (strain RM20B and RM23A) were classified as phylogenetic type D₂ and D₁, respectively.
206 Phylotype D₁ and D₂ include intra-intestinal pathogenic strains (Escobar-Páramo et al., 2004), thus
207 those multidrug-resistant D₁ and D₂ *E. coli* can be a risk factor for serious food-poisoning. In
208 addition, 6 isolates seemed to encode *bla*_{TEM} gene on the same plasmid with *tet* genes and/or *sul*
209 genes because those resistance genes were transferred to its recipients simultaneously. The spread of
210 AMP-TET-SXT-resistant *E. coli* was detected at fecal isolates from Ugandan livestock (Okubo et al.,
211 2019). These results suggest that multidrug-resistant *E. coli* can be spread from food-producing
212 animals to consumers via retail meats.

213 The spread of bacteria with resistance to clinically important antimicrobial agents, such as
214 ESBL-producing *E. coli*, is a global concern. Several review papers reported that ARB, especially
215 ESBL-producing *Enterobacteriaceae*, in livestock is a risk factor to human health because these
216 bacteria can be spread from farms to consumers via the food chain (Economou and Gousia, 2015;
217 Founou et al., 2016). Among our isolates, no cephalosporin-resistant *E. coli*, which are suspected to
218 be ESBL-producers, were detected from retail meats. A previous cross-sectional study on *E. coli*
219 derived from dairy cattle in Uganda reported that only 4 of 385 *E. coli* isolates (1.03%) were
220 identified as ESBL-producers harboring *bla*_{CTX-M-15} or *bla*_{CTX-M-27} genes (Ball et al., 2019). In
221 contrast, a previous report from a national hospital in Uganda reported that 59.7% (28/42) of *E. coli*
222 derived from blood specimens were resistant to CTX, and 70.2% (33/42) were resistant to
223 ceftriaxone (Kajumbula et al., 2018). Another study in a rural area of Uganda reported that 11.8% of
224 *E. coli* and *Klebsiella pneumoniae* from clinical stool samples (n = 21) showed resistance to the 3rd
225 and 4th generation cephalosporins (Stanley et al., 2018). The gap in frequency of
226 cephalosporin-resistant *E. coli* between Ugandan animal-related samples and human samples
227 suggests that ESBL-producing *E. coli* do not originate from livestock or retail meats but from human
228 communities in Uganda.

In conclusion, we detected antimicrobial-resistant *E. coli* from retail meat from roadside butcheries in Uganda. Our results showed that more than 90% of the retail meats at roadside butcheries were contaminated with fecal-associated *E. coli*, and some were resistant to AMP, TET, and/or SXT. These findings suggest that ARB in livestock can easily be transferred to humans through the food chain. Little attentions appeared to be paid for hygiene conditions in those roadside butcheries. Thus, sanitary control is important for protecting consumers from foodborne diseases and preventing the spread of ARB. But it is not realistic to regulate or restrict all the roadside butcheries. Instead, we need to reduce ARB at the farm level. We considered that the prudent use of antimicrobials in food-producing animals is the most crucial thing to stop the further emergence and dissemination of ARB.

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259 **Disclosure statement**

260 None declared.

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263 **Ethical approval**

264 Not required.

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352

353 **Figure legends**

354

355 Figure 1. Sampling and processing of retail meat samples. Retail meat samples were purchased from
356 roadside butcherries (a) in Kampala, Uganda. The meat surfaces were swabbed (b) and suspended in
357 saline solution. The suspension was inoculated on a PetriFilm SEC plate (c) to isolate *E. coli* (blue
358 colonies).

359

360 Figure 2. Frequency of resistant isolates (n = 89) to respective antimicrobial agents. Abbreviations:
361 AMP, ampicillin; GEN, gentamicin; TET, tetracycline; NAL, nalidixic acid; CIP, ciprofloxacin; CHL,
362 chloramphenicol; SXT, sulfamethoxazole-trimethoprim.

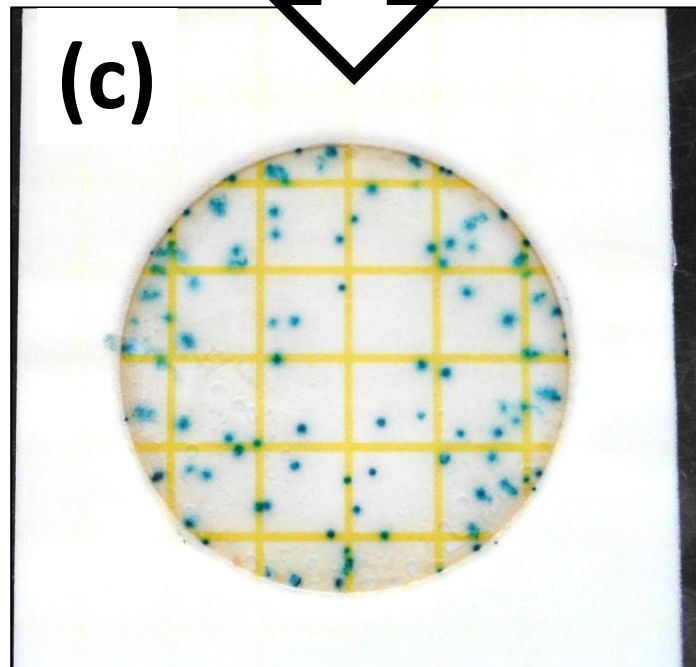
(a)



(b)



(c)



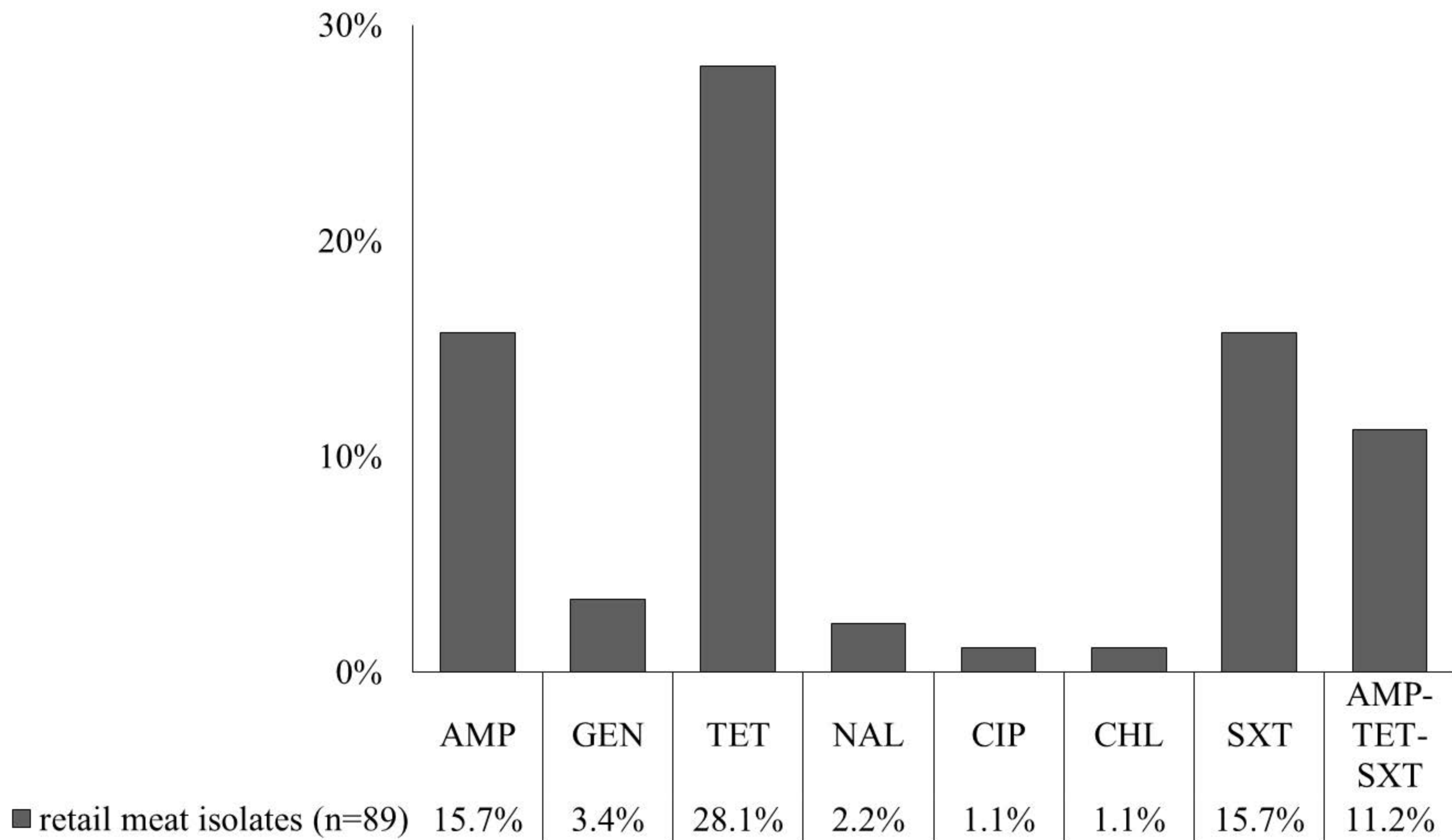


Table 1. Transfer frequency of multidrug-resistant *E. coli* from retail meats (n = 10) and antimicrobial susceptibility of those donors and recipients. All donors in this table were isolated from beef ribs.

Isolate name	Phylogenetic type	MIC of donor (mg/L)			Resistance genes in donor	Transfer frequency (Resistance to AMP)	Resistance genes in transconjugant
		AMP	TET	SXT			
RM04A	A ₁	> 128	> 64	> 152/8	<i>bla</i> _{TEM} , <i>tetA</i> , <i>tetB</i> , <i>sul1</i> , <i>sul2</i>	$5.13 \times 10^{-3} \pm 4.29 \times 10^{-3}$	<i>bla</i> _{TEM} , <i>tetA</i> , <i>tetB</i> , <i>sul1</i> , <i>sul2</i>
RM13B	B1	> 128	> 64	> 152/8	<i>bla</i> _{TEM} , <i>tetA</i> , <i>tetB</i> , <i>sul1</i> , <i>sul2</i>	$7.31 \times 10^{-3} \pm 5.32 \times 10^{-3}$	<i>bla</i> _{TEM} , <i>tetA</i> , <i>sul1</i> , <i>sul2</i>
RM16A	B1	> 128	64	> 152/8	<i>bla</i> _{TEM} , <i>tetA</i> , <i>sul1</i> , <i>sul2</i>	$< 1.96 \times 10^{-7}$	(no transconjugants)
RM16B	B1	128	64	> 152/8	<i>bla</i> _{TEM} , <i>tetA</i> , <i>sul2</i>	$< 5.56 \times 10^{-7}$	(no transconjugants)
RM20B	D ₂	> 128	> 64	> 152/8	<i>bla</i> _{TEM} , <i>sul2</i>	$2.03 \times 10^{-4} \pm 2.59 \times 10^{-4}$	<i>bla</i> _{TEM} , <i>sul2</i>
RM23A	D ₁	> 128	64	> 152/8	<i>bla</i> _{TEM} , <i>tetA</i> , <i>sul1</i> , <i>sul2</i>	$< 1.52 \times 10^{-7}$	(no transconjugants)
RM23B	A ₁	> 128	> 64	> 152/8	<i>bla</i> _{TEM} , <i>tetA</i> , <i>sul1</i> , <i>sul2</i>	$4.92 \times 10^{-3} \pm 6.13 \times 10^{-3}$	<i>bla</i> _{TEM} , <i>tetA</i> , <i>sul1</i> , <i>sul2</i>
RM27B	B1	> 128	> 64	> 152/8	<i>bla</i> _{TEM} , <i>tetA</i> , <i>sul2</i>	$1.81 \times 10^{-4} \pm 1.58 \times 10^{-4}$	<i>bla</i> _{TEM} , <i>tetA</i> , <i>sul2</i>
RM28B	B1	> 128	> 64	> 152/8	<i>bla</i> _{TEM} , <i>tetA</i> , <i>sul2</i>	$3.27 \times 10^{-4} \pm 4.03 \times 10^{-4}$	<i>bla</i> _{TEM} , <i>tetA</i> , <i>sul2</i>
RM56B	B1	> 128	64	> 152/8	<i>bla</i> _{TEM} , <i>tetA</i> , <i>sul1</i> , <i>sul2</i>	$< 7.75 \times 10^{-8}$	(no transconjugants)

Abbreviations. AMP, ampicillin; TET, tetracycline; SXT, sulfamethoxazole-trimethoprim