Isolation and characterization of antimicrobial-resistant *Escherichia coli* from retail meats from roadside butcheries 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: A_0, A_1, B_1, $B2_2$, $B2_3$, D_1, and D_2 (Escobar-Páramo et al., 2004). *E. coli* belonging to groups A_0, A_1, and B_1 are usually commensal and nonpathogenic strains. Groups $B2_2$ and $B2_3$ include extra-intestinal pathogenic strains related to urinary tract infections, and groups D_1 and D_2 include intra-intestinal pathogenic strains.

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

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

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

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

Results

We isolated 103 *E. coli* isolates from 58 retail meat samples (58/64; 90.6%). Among them, 14 had the same phylogenetic type and antimicrobial susceptibility pattern and were thus suspected to be duplicated clones and omitted from further analysis. Therefore, we used 89 *E. coli* isolates in this study. The phylogenetic patterns of retail meat isolates were as follows: A_0, 4.5% (4/89); A_1, 7.9% (7/89); B1, 70.8% (63/89); $B2_2$, 2.2% (2/89); $B2_3$, undetected; D_1, 13.5% (12/89); and D_2, 1.1% (1/89).

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

All AMP-resistant retail meat isolates (14/14) harbored the bla_{TEM} gene. TET-resistant retail meat isolates harbored several *tet* genes: *tetA*, 68.0% (17/25); *tetB*, 32.0% (8/25); *tetE*, 4.0% (1/25); and *tetG*, 4.0% (1/25). Two isolates harbored both *tetA* and *tetB*. The most prevalent SXT-resistance 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 butcheries 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 butcheries (Fig. 1) or during transportation, resulting in a high prevalence of *E. coli* on the meat surfaces.

PCR-based phylogenetic typing showed that group B1 was the most frequently detected phylotype from retail meats (70.8%), followed by groups D_1 (13.5%) and A_0 (7.9%). *E. coli* groups A_0, A_1, and B1 are commensal strains that include non- or low-pathogenic *E. coli* in the animal digestive tract (Carlos et al., 2010). The high prevalence of *E. coli* group B1 from retail meats is consistent with a previous report that group B1 was mainly isolated from the feces of herbivorous animals, such as cows, and in retail meats (Carlos et al., 2010; Johnson et al., 2009). In addition, our previous study reported that phylotype B1 was also prevalent among fecal *E. coli* isolates from Ugandan livestock (Okubo et al., 2019). In agreement with those reports, our results suggest that *E. coli* in the livestock digestive tract can be distributed from farms to consumers directly via retail meats, especially under the conditions of a poor temperature-controlled supply chain.

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

As for multidrug-resistance, 10 isolates showed AMP-TET-SXT resistance. Among them, 2 isolates (strain RM20B and RM23A) were classified as phylogenetic type D_2 and D_1, respectively. Phylotype D_1 and D_2 include intra-intestinal pathogenic strains (Escobar-Páramo et al., 2004), thus those multidrug-resistant D_1 and D_2 E. coli can be a risk factor for serious food-poisoning. In addition, 6 isolates seemed to encode bla_{TEM} gene on the same plasmid with *tet* genes and/or *sul* genes because those resistance genes were transferred to its recipients simultaneously. The spread of AMP-TET-SXT-resistant *E. coli* was detected at fecal isolates from Ugandan livestock (Okubo et al., 2019). These results suggest that multidrug-resistant *E. coli* can be spread from food-producing animals to consumers via retail meats.

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

None declared.

Ethical approval

Not required.

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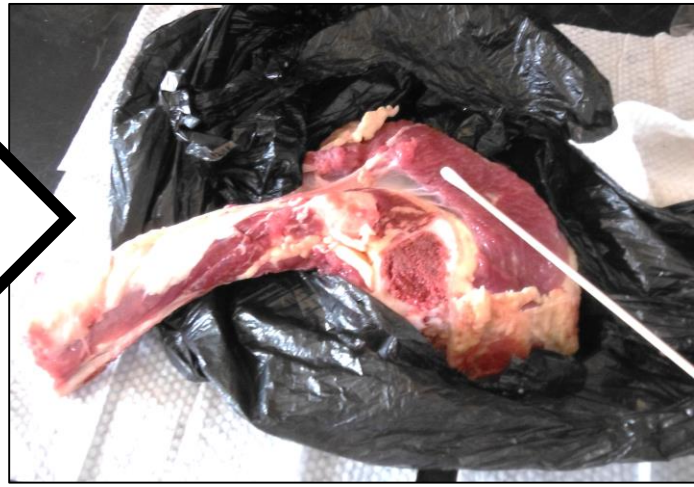
352
353 **Figure legends**

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

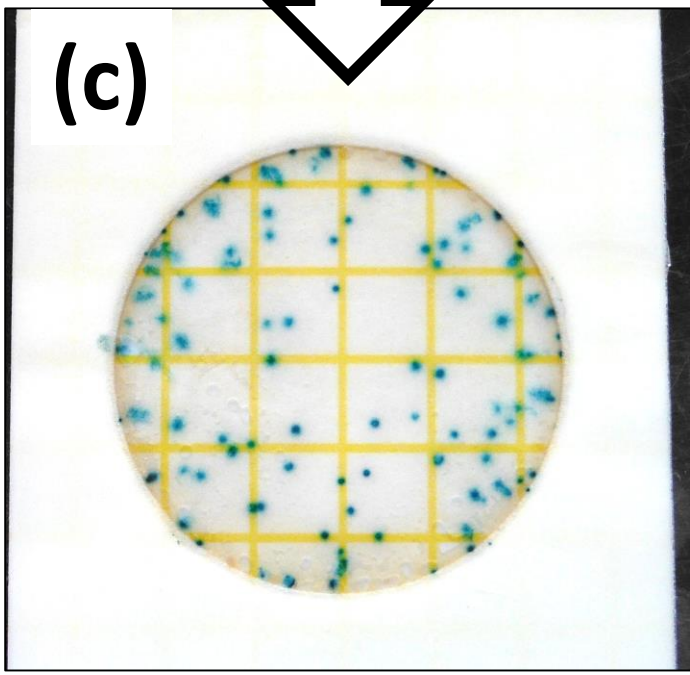
Figure 2. Frequency of resistant isolates (n = 89) to respective antimicrobial agents. Abbreviations: AMP, ampicillin; GEN, gentamicin; TET, tetracycline; NAL, nalidixic acid; CIP, ciprofloxacin; CHL, chloramphenicol; SXT, sulfamethoxazole-trimethoprim.

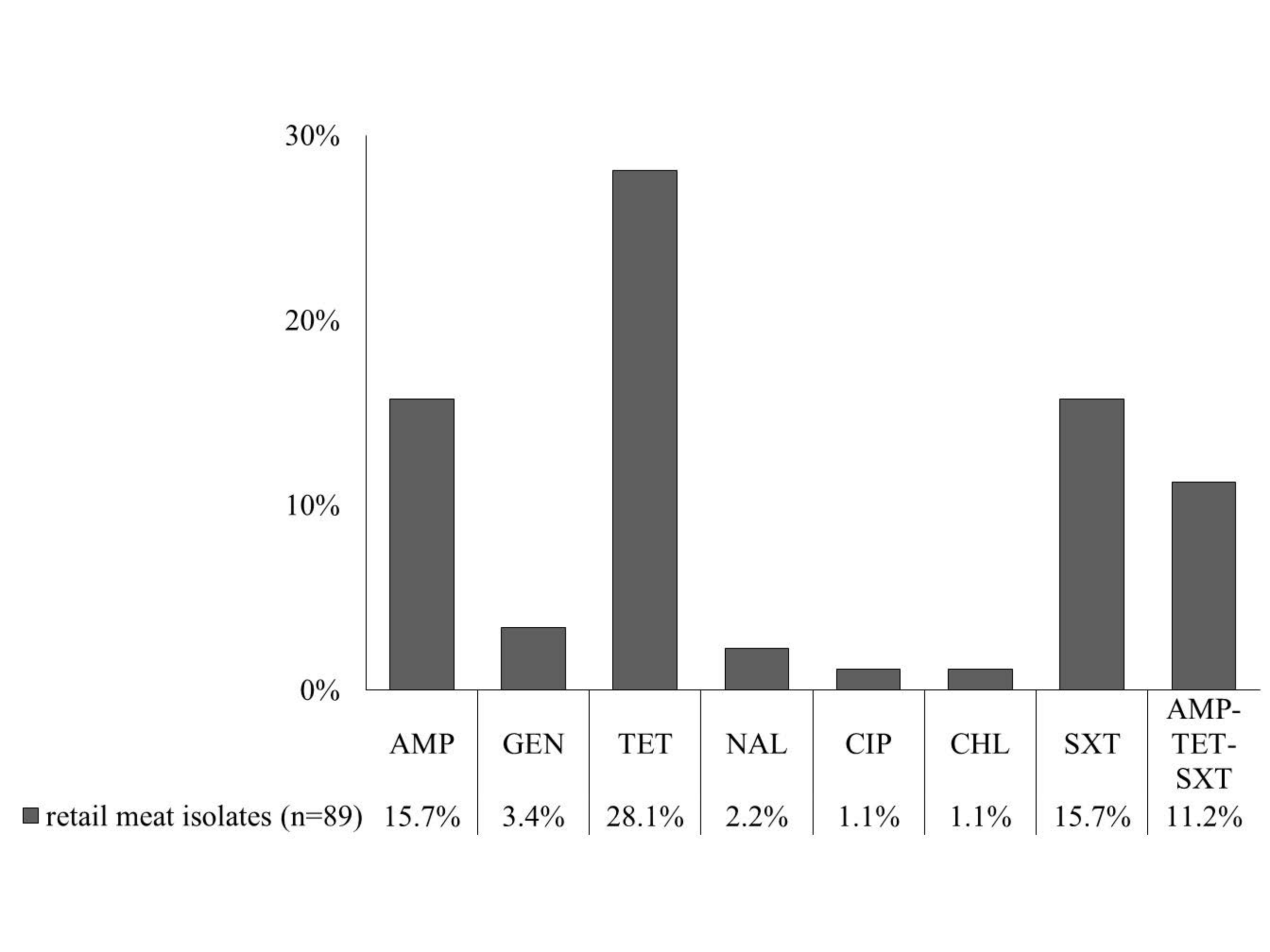
(a)

(b)



(c)




30%
20%
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AMP
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SXT
AMP-TET-SXT
retail meat isolates (n=89)
15.7%
3.4%
28.1%
2.2%
1.1%
1.1%
15.7%
11.2%

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	*bla*$_{TEM}$, *tetA*, *tetB*, *sul1*, *sul2*	$5.13 \times 10^{-3} \pm 4.29 \times 10^{-3}$	*bla*$_{TEM}$, *tetA*, *tetB*, *sul1*, *sul2*
RM13B	B1	> 128	> 64	> 152/8	*bla*$_{TEM}$, *tetA*, *tetB*, *sul1*, *sul2*	$7.31 \times 10^{-3} \pm 5.32 \times 10^{-3}$	*bla*$_{TEM}$, *tetA*, *sul1*, *sul2*
RM16A	B1	> 128	64	> 152/8	*bla*$_{TEM}$, *tetA*, *sul1*, *sul2*	$< 1.96 \times 10^{-7}$	(no transconjugants)
RM16B	B1	128	64	> 152/8	*bla*$_{TEM}$, *tetA*, *sul2*	$< 5.56 \times 10^{-7}$	(no transconjugants)
RM20B	D_2	> 128	> 64	> 152/8	*bla*$_{TEM}$, *sul2*	$2.03 \times 10^{-4} \pm 2.59 \times 10^{-4}$	*bla*$_{TEM}$, *sul2*
RM23A	D_1	> 128	64	> 152/8	*bla*$_{TEM}$, *tetA*, *sul1*, *sul2*	$< 1.52 \times 10^{-7}$	(no transconjugants)
RM23B	A_1	> 128	> 64	> 152/8	*bla*$_{TEM}$, *tetA*, *sul1*, *sul2*	$4.92 \times 10^{-3} \pm 6.13 \times 10^{-3}$	*bla*$_{TEM}$, *tetA*, *sul1*, *sul2*
RM27B	B1	> 128	> 64	> 152/8	*bla*$_{TEM}$, *tetA*, *sul2*	$1.81 \times 10^{-4} \pm 1.58 \times 10^{-4}$	*bla*$_{TEM}$, *tetA*, *sul2*
RM28B	B1	> 128	> 64	> 152/8	*bla*$_{TEM}$, *tetA*, *sul2*	$3.27 \times 10^{-4} \pm 4.03 \times 10^{-4}$	*bla*$_{TEM}$, *tetA*, *sul2*
RM56B	B1	> 128	64	> 152/8	*bla*$_{TEM}$, *tetA*, *sul1*, *sul2*	$< 7.75 \times 10^{-8}$	(no transconjugants)

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