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Antibiotic Residues in Milk from Three Popular Kenyan Milk Vending Machines

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Abstract. Milk vending machines (MVMs) are growing in popularity in Kenya and worldwide. Milk vending machines dispense varying quantities of locally sourced, pasteurized milk. The Kenya Dairy Board has a regulatory framework, but surveillance is weak because of several factors. Milk vending machines' milk is not routinely screened for antibiotics, thereby increasing potential for antibiotic misuse. To investigate, a total of 80 milk samples from four commercial providers (*N* = 25), street vendors (*N* = 21), and three MVMs (*N* = 34) were collected and screened in Eldoret, Kenya. Antibiotic residue surveillance occurred during December 2016 and January 2017 using Idexx SNAP tests for tetracyclines, sulfamethazine, beta-lactams, and gentamicin. Overall, 24% of MVM samples and 24% of street vendor samples were presumably positive for at least one antibiotic. No commercial samples were positive. Research into cost-effective screening methods and increased monitoring by food safety agencies are needed to uphold HAACP for improving antibiotic stewardship throughout the Kenyan private dairy industry.

Beyond the spread of antimicrobial resistance in the hospital environment, enhanced understanding of veterinary and environmental health factors (e.g., antibiotic stewardship in agriculture, improved sanitation and hygiene, etc.) are needed to slow or reverse the rapid and global spread of antibiotic-resistant bacteria.[1] The greatest concerns for antibiotic resistance (AR) development are arising in low- and middle-income nations, where science is lacking important quantitative data from these high-risk areas for AR development.[1] Globally, research needs of AR are great in Africa, particularly because of limited resources for discouraging antibiotic misuse in clinic, home, and farm settings.[2–5] Antibiotic resistance is a problem without borders as AR genes can be transmitted rapidly from animal-associated flora (e.g., manure bacteria) to nonpathogenic or pathogenic flora in the environment. Such AR bacteria may then be acquired and shed by livestock, wildlife, or humans that travel.[6]

In East Africa, the role of cattle and cattle antibiotics for promoting human-acquired AR has gained great attention among medical, veterinary, and global public health communities.[6,7] In rural and peri-urban areas of Kenya, humans and livestock are in close proximity daily and may share drinking water sources. Animal-related fecal contamination of water, soil, and food supplies with AR flora may be possible in the presence of antibiotics.[7] Among these and several other reasons, a recent U.K. report warned that if unchecked, antimicrobial usage will result in AR infections being the world's leading killer by the year 2050 with the most significant AR-related mortality anticipated to occur in Africa.[8]

Because locally sourced milk is routinely produced and consumed in Kenya, milk has been a focus of research investigations since the early 2000s when antibiotic use became widespread and increased in the dairy industry for boosting agricultural productivity.[9] Such investigations produced an increased regulation of antibiotics among large facilities producing wholesale milk for regional markets in East Africa. In 2006, the East African Standard (EAS) for pasteurized milk[10] was established, thereby requiring regional milk sellers to comply with the maximum residue limits (MRLs) for veterinary drugs from the Codex Alimentarius.[11] Smaller producers remain a cause for concern as evidenced in the frequency of multidrug-resistant *Staphylococcus aureus*, which are observed in small farm milk samples at a rate double that of Kenya's large farm rate.[12] The issue has become of greater concern with the development of milk vending machines (MVMs). Milk vending machines are not automated teller machines (ATMs); however, locally, they are referred to as "Milk ATMs." Milk vending machines are gaining popularity in low- and middle-income nations worldwide. In Kenya, MVMs are often advertised as providing locally sourced milk from small farmers. Many supermarkets in Kenya have an MVM at the storefront where consumers may purchase milk in various quantities dispensed in volumes as small as 0.25 L. Government regulators in Kenya are not currently examining milk from these MVMs for antibiotic residues. The Ministry of Agriculture, Livestock, and Fisheries is striving to address deficiencies in the national dairy program for local sellers.[13] Commercial providers adhere to the EAS[10] as they may export product throughout East Africa.

The widespread growth of MVMs in Uasin Gishu County warranted the study. In January 2016, in a meeting with county officials pertaining to rural household water quality, county officials independently raised concerns regarding MVMs and the potential for antibiotic residues. We therefore hypothesized that the prevalence of detectable antibiotic residues in MVM milk would be higher than in commercially packaged milk from the same supermarkets housing the MVMs. Given a recent Kenyan study demonstrated 44% of milk samples from smallholder collections in adjacent counties contained beta-lactam antibiotic residues,[14] we expected to find positive detections in MVM samples.

For screening for elevated levels of antibiotic residues in milk from MVMs, samples were collected in sterile Nasco (Fort Atkison, WI) Whirl-Pak containers from Eldoret, located in western Kenya (Uasin Gishu County). Samples were also collected in Whirl-Paks from street vendors. Prepackaged commercial samples were collected from four different brands at the supermarket. The samples were collected at least 2 days apart from each location during the months of December 2016 and January 2017. All samples were transported in a cooler and testing occurred within 4 hours of collection.

IDEXX (Westbrook, ME) SNAP® tests were used in accordance with the manufacturer's instruction for screening for elevated levels of beta-lactams, sulfamethazine, tetracyclines,

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Table 1

Comparison of residue detection thresholds for SNAP tests used in this study vs. the MRLs for each antibiotic as presented in the July 2017 Codex Alimentarius[11]

SNAP test	Agent	Detection level (ppb)	Codex MRLs (ppb)
NBL beta-lactams	Amoxicillin	≤ 8	4
	Ampicillin	≤ 6	
	Ceftioufur	≤ 20	100
	Cephapirin	≤ 12	
	Penicillin	≤ 4	4
Sulfamethazine	Sulfamethazine	≤ 10	25
Tetracycline	Tetracycline	≤ 50	100*
	Chlortetracycline	≤ 100	100*
	Oxytetracycline	≤ 50	100*
Gentamicin	Gentamicin	≤ 30	200

MRLs = maximum residue levels.
* Combined totals of all three in the tetracycline group should not exceed 100 ppb.

and gentamicin. Each SNAP test device is designed as a single-use, field-portable enzyme-linked immunosorbant assay, which allowed for ease of use in the region.[15] The residue detection thresholds of the SNAP tests relate to the manufacturer's design for compliance monitoring that meets or exceeds the U.S. Food and Drug Administration's (FDA) protocols for evaluating FDA established tolerance, safe, and/or action levels. Sample results were recorded as positive or negative using the color indicator unique for each test. Although presumably positive by the SNAP test, Table 1 demonstrates the potential for some positive samples to exceed the U.S. criteria while being below MRLs defined in the Codex Alimentarius as an international food standard. For drawing greater conclusions among presumably positive samples, an analytical analysis would be required to obtain true concentrations.

Overall, 80 samples were collected with most coming from three MVMs (N = 34) (Table 2. For MVMs and street vendors, positive results were periodically observed. However, no prepackaged commercial milk samples tested positive for any of the antibiotic residues evaluated (Table 2). In regard to MVMs, 24% of samples had a positive result for at least one antibiotic. Similarly, 24% of samples from unique street vendors tested positive for at least one antibiotic (Table 2). No sample tested positive for more than one antibiotic.

Fisher's exact tests were used to determine if significant differences existed between the frequencies of any antibiotic detection in milk from the MVMs (8/34), commercially packaged milk (0/25), and street vendor milk (5/21). In comparing antibiotic residue prevalence in MVMs versus commercially packaged milk, there was a significant difference (P = 0.016). Similarly, there was a significant difference in antibiotic residue prevalence between street vendor milk samples and the commercially packaged milk (P = 0.015). There was no difference in the frequency of milk samples that tested positive for any antibiotic between MVM and street vendor samples (P = 0.614).

Among MVMs, there was a difference in the prevalence of MVMs with a positive antibiotic detection. Specifically, 45% of MVM samples (5 of 11) from MVM-1 were positive for at least one antibiotic residue (Table 3), which was significantly higher than the zero of 12 from MVM-2 (P = 0.014). Among positive MVM and street vendor samples, tetracycline and gentamicin were most prevalent. For MVM-1, three separate antibiotics were found across 11 samples over 24 sample days. Table 3 illustrates that one MVM (MVM-1) may contain multiple residues during a relatively short period.

The results presented here are limited, as positive results were not further evaluated with analytical chemistry techniques. In addition, with regard to tetracycline, the screening method sensitivity used here was at 50 ppb rather than 100 ppb (Table 1), and positive detections of tetracyclines in Kenyan milk by screening are not always substantiated after analytical laboratory evaluation.[16] It is noteworthy that no commercial samples tested positive using the SNAP screening methods.

Overall, observing antibiotic residues in milk is not unexpected in Kenya or abroad.[9,17,18] Noteworthy here is the emerging, rapidly expanding, understudied MVM concept in Kenya and abroad. For the growing MVM supply chain, our results indicate a greater need for further investigating Kenyan MVMs and antibiotic usage in local milk supply chains. Recently (2017), in nearby Nakuru County, 28.8% (23/80) of milk samples from peri-urban areas were positive for antibiotic residues.[16] Among our noncommercial samples (MVM + street vendor) in Eldoret, we observed 23.6% (13/55) positive.

As invasive antibiotic-resistant *Salmonella* disease rises at alarming rates in sub-Saharan and East Africa,[19] along with AR in other pathogens of clinical relevance,[20] enhanced efforts to mitigate and prevent AR in the environment are needed. Priority actions are needed for upholding EAS pasteurized milk specifications, which include the Codex Alimentarius MRLs, for local milk. Such priorities have been identified in the National Dairy Development Policy including procuring reliable testing equipment, providing testing training, sensitizing stakeholders on the importance of appropriate antibiotic use, and enforcement of standards.[13]

In Kenya, current food safety regulations remain fragmented over four agencies. Coordination and surveillance remain challenging. Milk seller's income also limits stakeholder engagement. Research into cost-effective screening methods and incentivizing MVM owner education may help. Kenyan regulators may benefit from developing a general decision tree for evaluating antibiotic residues, which would identify different options for hazard identification, hazard characterization, and exposure assessment.

The present study is limited. Analytical techniques were not used to confirm if residue concentrations exceeded MRLs. In addition, MVMs were replenished multiple times daily because of their popularity, but common suppliers of the units or

Table 2

Prevalence of detectable levels of antibiotic residues in milk for the various sample types from Eldoret, Kenya

	Tetracyclines	Sulfamethazine	Beta-lactams	Gentamicin	Any antibiotic
Sample type	± (%)	± (%)	± (%)	± (%)	± (%)
Commercial	0/25 (0)	0/25 (0)	0/25 (0)	0/25 (0)	0/25 (0)
Milk MVM	2/34 (5.9)	1/34 (2.9)	2/34 (5.9)	3/34 (8.8)	8/34 (24)
Street vendor	3/21 (14)	0/21 (0)	0/21 (0)	2/21 (9.5)	5/21 (24)

TABLE 3
Prevalence of detectable levels of antibiotic residues in milk for the individual commercial brands and MVMs evaluated in Eldoret, Kenya

Sample type	Tetracyclines ± (%)	Sulfamethazine ± (%)	Beta-lactams ± (%)	Gentamicin ± (%)	Any antibiotic ± (%)
Commercial 1	0/7 (0)	0/7 (0)	0/7 (0)	0/7 (0)	0/7 (0)
Commercial 2	0/6 (0)	0/6 (0)	0/6 (0)	0/6 (0)	0/6 (0)
Commercial 3	0/7 (0)	0/7 (0)	0/7 (0)	0/7 (0)	0/7 (0)
Commercial 4	0/5 (0)	0/5 (0)	0/5 (0)	0/5 (0)	0/5 (0)
MVM-1	2/11 (18)	1/11 (9.1)	0/11 (0)	2/11 (18)	5/11 (45)
MVM-2	0/12 (0)	0/12 (0)	0/12 (0)	0/12 (0)	0/12 (0)
MVM-3	0/11 (0)	0/11 (0)	2/11 (18)	1/11 (9.1)	3/11 (8.8)

MVMs = milk vending machines.

incomplete removal of milk could contaminate bulk samples with detectable levels of antibiotic residue. Additional research and surveillance pertaining to MVMs is recommended for identifying the sources of antibiotic entry into the supply chain. Overall, given MVMs are growing in popularity, further investigations and surveillance are strongly encouraged.

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