

ANTIMICROBIAL AGENTS DETECTED IN MARKETED MILK IN KENYA

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Summary

Drug residues in foods are a major public health concern in many countries, especially where most food sales bypass official quality assurance channels. In common with many tropical countries, sales of unpasteurized milk in Kenya account for over 85% of marketed milk. This milk is either sold directly from producers to consumers or via various cadres of informal market agents. Besides residues that may arise from lack of adherence to withdrawal times following cow therapy, there have been concerns that some antimicrobial agents may be added to informally marketed milk to extend its shelf life.

As part of a large study to assess public health hazards associated with marketed milk, samples were collected seasonally between January 1999 and January 2000 from raw (unpasteurized) milk consuming households and informal market agents of various cadres. Pasteurised milk samples were also collected from retail points and tested for comparison. All samples were screened for antimicrobial residues using charm AIM-96 and Charm-ROSA (Charm Sciences Inc, USA) tests. The former detects a wide range of anti-microbials, and the latter detects β -lactams and tetracyclines specifically, at levels above maximum residue limits (MRLS) recommended by the European Union (EU). The Charm-AIM screening test showed that 9.4% and 5.7% of samples from consumer households and market agents had antimicrobial residues above EU MRLS, respectively. It was concluded that antimicrobial residues were more likely to have originated at farm-level than because of poor market handling practices.

Key words: *Anti-microbial residues, marketed milk, Kenya.*

Introduction

Antimicrobial agents in milk are undesirable because they cause hypersensitivity (Osion and Sanders, 1975), drug resistance (Nijsten *et al.*, 1996) and specific tissue damage (Schultz *et al.*, 1963; Moffit *et al.*, 1974) in humans. They also inhibit organisms required in the processing of cultured milk products. In Kenya anti-microbial agents of aminoglycosides, β -lactams, sulfonamides and tetracyclines are used extensively for treatment of livestock diseases. Anti-microbial residues have been reported in milk following all routes of administration (Suliman *et al.*, 1990; Roudant *et al.*, 1990) and ingestion of contaminated feed (McEvoy *et al.*, 2000). Penicillin residues have been demonstrated in 1.2% of milk deliveries at Kenya Co-operative Creameries (Chewulukei (1978) and general veterinary drugs have been found in slaughter-house meat (Mdachi and Murilla, 1991 and Muriuki, 1992).

Since market liberalisation in 1992, the proportion of raw milk sold in urban centres has markedly increased, thereby raising public health concerns (Omire *et al.*, 1999). Besides residues that may result from lack of adherence to withdrawal times following therapy, there have been concerns that some antimicrobial agents may be added to informally marketed milk to extend its shelf life. This paper describes the use of Charm AIM-96 and Charm-ROSA (Charm Sciences Inc, USA) kits to test for antimicrobial agents in milk informally and formally marketed by various market agents in Kenya

Materials and Methods

As part of a large study to assess public health hazards associated with marketed milk, samples were collected between January 1999 and January 2000 from 212 and 222 raw (unpasteurized) milk consuming households in the dry and wet season, respectively. At the market-level, 262 and 246 informal market agents were interviewed and milk samples collected from them during the two

respective seasons. Respondents were randomly selected within production system (extensive and intensive) and human population density (urban, peri-urban and rural) strata. Nakuru and Narok districts represented extensive production systems and low population density (also medium market access). Nairobi and Kiambu Districts represented intensive production systems and high population density (also high market access). The informal market agents that were sampled included dairy co-operatives, milk bars, milk shops and mobile traders on foot, bicycle or motorised transport. Attempts were made during the wet season to sample the same agent sampled in the dry season. Where this was not possible, substitution was done within the same locality. A total of 110 formally (pasteurized) marketed milk samples from Nairobi and Nakuru were also tested.

All samples were screened using the Charm AIM-96 anti-microbial inhibition assay screening kit (Charm Sciences Inc., USA) according to manufacturer's recommendations. The test kit detects β -lactams, tetracyclines, aminoglycosides, macrolides and sulphonamides at levels above maximum residue limits (MRLs) recommended by the EU (detection levels and EU MRLs for the two antibiotics commonly used in Kenya, penicillin G and oxytetracycline, are 4ppb and 100ppb, respectively). Briefly, 50 μ l of each sample was added in duplicate to the supplied microtitre plate followed by 200 μ l of a mixture of *Bacillus stearotherophilus* spore tablet and lyophilised medium dissolved in 22mls of deionized water. The plate was then sealed and tightly secured by screws and incubated for 3-4 hours. Positive and negative controls were also included in the assay. The positive milk control consisted of antibiotic free milk determined using *Micrococcus lutea* inhibition assay mixed with penicillin G standard or sulfamethazine standard. To 50 μ l of the positive control milk, 200 μ l of bacterial spore and lyophilised media was added. The negative control consisted of 50 μ l of negative control tablet dissolved in distilled water and 200 μ l of the test bacteria and media dissolved in deionized water. Test results were read using colour contrasts and scored from 1-5 (negative = 1-3 and positive = 4-5).

All samples tested positive by the Charm-AIM kit were subsequently analysed using the new United States Food and Drug Administration approved Charm-ROSA (Charm Sciences Inc., USA) test to identify specifically those containing β -lactams and tetracyclines. Lower detection limits for the Charm-ROSA kit are 2ppb and 125ppb for penicillin G and oxy-tetracycline, respectively. In addition, results from the two tests were experimentally compared by testing milk samples from eight lactating dairy cows injected with therapeutic doses of intra-mammary and intra-muscular preparations of Penicillin G and 10% oxytetracycline. One pre-treatment and five post-treatment milk samples were collected daily from the eight cows and tested.

Results and Discussion

Overall, 37 (9.4%) and 27 (5.7%) of consumer- and market-level samples, respectively, were positive on the Charm AIM test (Table 1). The proportion of consumer-level samples from rural areas with antibiotic residues were three times those from urban areas. Among informal market level samples, the number with residues decreased with increasing levels of bulking with milk bars and small milk traders having much higher proportion of samples with anti-microbials compared to samples from dairy co-operatives. Nine out of 110 (8.2%) pasteurised milk samples had residues.

Table 1. Numbers and proportions of consumer- and market-level samples testing positive for anti-microbials on Charm AIM test in both seasons.

Source of sample	Number	%
<i>Consumer households</i>		
Urban consumers (Nairobi and Nakuru)	8	4.0
Rural consumers (Nakuru)	29	15.0
<i>Informal market agents in high market access and intensive production area (Nairobi/Kiambu)</i>		
Coops/collection centres centres/Self-help groups	1	1.5
Milk Bars	10	9.4
Milk Shops/kiosks	5	5.5
Small mobile traders	4	7.1
<i>Informal market agents in medium market access and extensive production area (Nakuru/Narok)</i>		
Coops/collection centres centres/Self-help groups	0	0
Milk Bars	2	3.8

Milk Shops/Kiosks	0	0
Small mobile traders	5	10.0
<i>Pasteurised milk in Nairobi & Nakuru</i>	9	8.2

The higher proportion of consumer-level milk samples with anti-microbial residues as detected by Charm-AIM test kit would imply that the residues are more likely to originate at the farm-level than because of bad market-level practices. On the other hand, the increased residues as milk moves up the market chain and bulking occurs (including of pasteurised milk) seems to suggest that anti-microbial agents may be added after the first milk sale transaction. Further investigation, including any dilution effects on anti-microbial residue levels, need further investigation.

These apparent high levels of antibiotic residues in marketed milk as detected by the Charm-AIM test need to be evaluated against the background of the results of the Charm-ROSA test and the experiment conducted to compare the two tests. None of the consumer- and market-level milk samples that were positive on the Charm-AIM test was positive on the Charm-ROSA test as well. And the Charm-AIM test classified as many as seven out of eight experimental samples as having Penicillin G or oxy-tetracycline residues up to the fifth day post-treatment, compared to the Charm-ROSA test that classified only one by the same day.

Whereas agreement between the two tests is inconclusive, these results indicate that the problem of anti-microbial residues in milk needs to be tackled at both the farm and market levels. To begin with, there is the need to define farm-level causal relationships to complement these data. Such information would be necessary to devise appropriate measures to reduce residues at both these levels.

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