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Short Communication: Assessment of tetracycline residue in local milk consumed in Yola, Adamawa State, Nigeria

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[2]Department of Science Laboratory Technology, School of Life Science, Modibbo Adama University Yola. P.M.B. 2076, Yola Adamawa State, Nigeria. Tel.: +234-90287690043, ♥email: alamiya@mau.edu.ng
[3]Modibbo Adama University Central Laboratory, Modibbo Adama University Yola. P.M.B. 2076, Yola Adamawa State, Nigeria
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Abstract. *Bello MI, Lamiya A, Hayatu IA, Zailani HA, Ja'afar JN, Raji ML, Abdullahi M. 2023. Short Communication: Assessment of tetracycline residue in local milk consumed in Yola, Adamawa State, Nigeria. Asian J Trop Biotechnol 20: 45-49.* Antimicrobial residues in food beyond the tolerance limit for human consumption will harm health, such as toxicity, allergy, teeth discoloration, teratogenicity, and, most importantly, antimicrobial resistance. Knowing the prevailing tetracycline usage pattern in cattle husbandry and the lack of information on tetracycline residue in the locally consumed milk in Yola Adamawa State, Nigeria, therefore, this study was conducted to assess the tetracycline residue in the region. One hundred and twenty-two local milk samples were collected through simple random sampling in the study area from local retailers. First, the samples were screened for general antimicrobial residue using the microbiological method. Antimicrobial-positive samples were then subjected to HPLC analysis to identify and quantify possible tetracycline residue. The results showed that 23% (28/122) were positive for antimicrobial residue, of which 68% (19/28) were identified as tetracycline. The overall tetracycline residue prevalence was 15.6% (19/122) with a mean concentration of 432.64 µg/L with the lowest and highest concentration of 52.91 µg/L and 1,597.29 µg/L, respectively. The proportion of tetracycline positive to negative samples differed significantly ($P<0.05$), occurring with probabilities of 0.16 and 0.84, respectively. This study revealed tetracycline at a low prevalence, but in most cases, 89.47% (17/19) at dangerous levels. Thus, there is a need to review the present antimicrobial regulatory mechanisms.

Keywords: Antibiotic, health hazard, prevalence, residue, tetracycline

INTRODUCTION

Milk is a nutritious food of animal origin that provides almost all the nutritional requirements of humans and animals (Kurjogi et al. 2019). It has been reported that Nigeria is the largest milk producer in West Africa and can potentially be the largest milk producer in Africa (Olatoye et al. 2016). In Nigeria, as in other African countries, most cattle husbandry is handled by the local trans-nomadic or semi-nomadic herdsmen with poor access to professional veterinary services (Olatoye et al. 2016). Therefore, promoting the indiscriminate use of antibiotics for therapeutic and prophylactic purposes (Olatoye and Ogundipe 2013; Alhaji et al. 2019) has led to the appearance of antimicrobial residue as either parent drug or metabolite in foods of animal origin (Oluwafemi et al. 2018). Annual Antimicrobial Usage (AMU) in food animals was estimated at 63,000 tons globally in 2015 and is projected to increase by about 67% by 2030 (Van Boeckel et al. 2015). Global top consumers of antimicrobials include China, the United States, and Brazil. However, a more than 200% relative increase has been projected in developing countries, with Myanmar, Indonesia, and Nigeria taking the lead (Alhaji et al. 2019). The antimicrobial residue in food has been implicative in toxicity (carcinogenicity, mutagenicity, nephropathy, hepatotoxicity, and bone marrow toxicity) and humans immunological responses (allergy) (Kabrite et al. 2019). Other implications include gut flora modification and increasing antimicrobial resistance (Olatoye and Ehinmowo 2010; Samandoulougou et al. 2015). These are health hazards to humans and animals. Milk sales from animals treated with antibiotics in developed countries have been banned until the withdrawal period is appropriately observed (Oluwafemi et al. 2018). Therefore, to ensure food safety, global regulatory authorities such as World Health Organization (1999) and the Food and Agricultural Organization (2008) have set Maximum Residue Limits (MRLs) and acceptable daily intake for several veterinary drugs in foods. The Food and Agricultural Organization, the World Health Organization (FAO/WHO), and the European Union (EU) have recommended a Maximum Residue Limit (MRL) of 100 µg/kg for tetracycline, oxytetracycline and chlortetracycline (singly or in combination) in milk.

Meanwhile, the US Food and Drug Administration (FDA) has set an upper legal level of 300 µg/kg for the combined residues of tetracycline, oxytetracycline, and chlortetracycline. The Joint Expert Committee on Food Additives has recommended an Acceptable Daily Intake (ADI) for tetracyclines residues at a concentration of 0-30 µg/kgBW/day (Aalipour et al. 2015). Studies revealed that tetracycline is one of the most commonly used antimicrobials in cattle husbandry in Northern Nigeria, and it persists in the body over a long period after administration (Kurjogi et al. 2019). Hence, there is the possibility that tetracycline's continuous passage through milk in lactating cows. Consumption of tetracycline-contaminated milk has been reported to have implications in primary and permanent teeth discoloration and pigmentation among infants and children under twelve due to short or long-term consumption (Alanazi et al. 2021). In addition, it has also been reported that tetracycline contamination poses the risk of teratogenicity when administered in the first trimester of pregnancy (Aalipour et al. 2015). Liu et al. (2017) also mentioned that consumption of tetracycline, even at a low concentration over a long period, can lead to the development of antibiotic resistance. Due to the limited information about residual antimicrobial levels in local milk consumed around Northern Nigeria, this work aimed to assess the tetracycline residue in local milk consumed in Yola Adamawa State, Nigeria.

MATERIALS AND METHODS

Study area

Yola, Northeastern Nigeria, is located on the Benue River at the latitude of 9°12'30.20" N and longitude of 12°28'53.26" E.

Sampling method and sample collection

Milk samples were collected from local milk retailers in Yola. One hundred and twenty-two (122) local milk samples were collected through simple random sampling in separate, sterile, well-labeled plastic containers. Samples were placed in an ice-cold box and transported to Chevron Biotechnology Center, Modibbo Adama University Yola, Nigeria, for analysis. The samples were stored at 4°C before being analyzed.

Screening test

Milk samples were screened for antimicrobial residue using the microbiological method described by Kurjogi et al. (2019) with some modifications (wells were used instead of sterile paper discs). *Bacillus subtilis* culture was grown in nutrient broth (Lifesave Biotech, USA) at 37°C for 16 hours. After overnight incubation, the culture was adjusted to 0.5 McFarland standard and inoculated on nutrient agar (Lifesave Biotech, USA) plates. Wells were bored on the Petri dish's surface using sterile micropipette tips. Then, 200 µL of the milk samples were dispensed into the wells and incubated at 37°C overnight. Zones of bacterial growth inhibition were measured in mm (Figure 1). In addition, a zone of inhibition with an annular diameter ≥ 1 mm was recorded as positive, and the results are presented in Table 1.

High-Performance Liquid Chromatography (HPLC) assay

The positive samples in the microbiological test were further analyzed using HPLC to identify and quantify tetracycline residue.

Preparation of standard solution

A standard solution was prepared as described by Marinou et al. (2019) as follows: 10 µg/mL stock solution was prepared by dissolving 100 µg of tetracycline analytical grade in 10mL methanol and refrigerated at 4°C. Working methanolic standards were subsequently prepared within the 0.08 -10 µg/mL concentration range.

Table 1. Antimicrobial residue detected in milk sample

Total sample size	Number/percentage of antimicrobial positive samples (Zone of inhibition with annular diameter ≥ 1mm)	Number/percentage of antimicrobial negative samples (Zone of inhibition with annular diameter < 1mm)
122	28/122 (23%)	94/122 (77%)

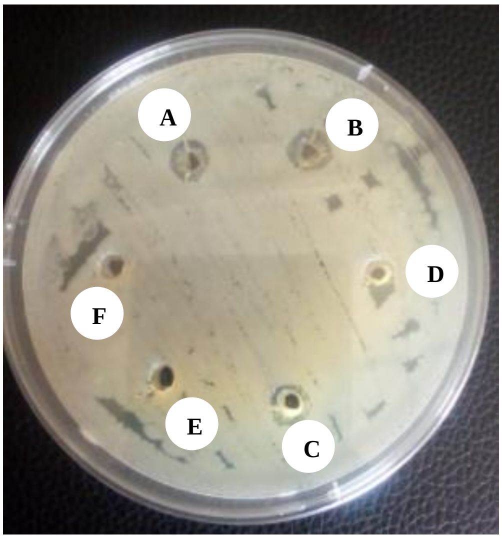

Figure 1. Antimicrobial assay for positive and negative milk samples based on inhibition zone. A, B, and C are positive antimicrobials; D, E, and F are samples with negative antimicrobials

Sample preparation

The sample preparation technique employed in this study is the optimized Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) technique as described in Marinou et al. (2019) work. According to the optimized protocol: an aliquot of 125 mg of QuEChERS material (25 mg of primary and secondary amines, 25mg of C18EC, and 75 mg of magnesium sulfate) was placed in a falcon tube with 1 mL methanol, 1 mL $C_2H_2O_4$ 0.01M and 0.5 g milk sample (stored at 4°C in the fridge). The sample was then vortexed for 30s and centrifuged at 3,500 rpm for 10 min. The supernatant was evaporated to dryness in a water bath at 40°C under a light stream of nitrogen. The dry residue was dissolved in 500 µL ultrapure water and filtered before the HPLC analysis.

Chromatographic conditioning

An orbit 100C_4 (µm, 250×4.0 mm) analytical column at ambient temperature separated the chemical compounds. The analytes were monitored at 355nm. The elution system consisted of 0.01M $C_2H_2O_4$ – 10^{-4}M Na_2EDTA/ACN, delivered at a low rate (0.9mL/minute), according to the gradient program: 0 min. 82:18v/v, 20min 60:40v/v. The dwell volume of the system used was 1.6mL. Inlet pressure was between 215 and 230 bar, and the injection volume was 100 µL.

Data analysis

Data obtained were analyzed using SPSS version 25. Student t-test was used to determine the significant difference ($P<0.05$) between positive and negative concentrations. In contrast, one-sample chi-square and one-sample binomial tests were used to analyze the proportion of positive to negative and the probabilities of their occurrences.

RESULTS AND DISCUSSION

Antimicrobial-positive samples were reported based on their ability to produce a zone of inhibition (≥1 mm annular diameter) on the nutrient agar plates (Figure 1). 23% (28/122) of the local milk samples were positive for antimicrobial residue Table 1. Furthermore, out of the 28 samples positive for antimicrobial residue, 19 (68%) were specific for tetracycline as confirmed by High Performance Liquid Chromatography (HPLC), constituting 15.6% (19/122) of the total milk samples in the study Table 2. The overall mean tetracycline residue concentration detected was 432.64 µg/L, with the lowest and highest concentrations at 52.91 µg/L and 1,597.29 µg/L, respectively. The standard calibration curve and HPLC chromatogram report of tetracycline-positive samples are presented in Figures 2 and 3, respectively.

Even though the proportion of tetracycline residue-positive samples reported in the present study was significantly lower than that of the negative ($P<0.05$), a high proportion of 89.47% (17/19) of tetracycline-positive samples had residue levels above the tolerance limits set by Codex Alimentarius Commission of 100 µg/L in milk.

Table 2. HPLC identification of tetracycline from antimicrobial positive samples

Total number of antimicrobial positive samples	Number/percentage of tetracycline positive samples	Number/percentage of tetracycline negative samples
28	19/28(68%)	9/28 (32%)

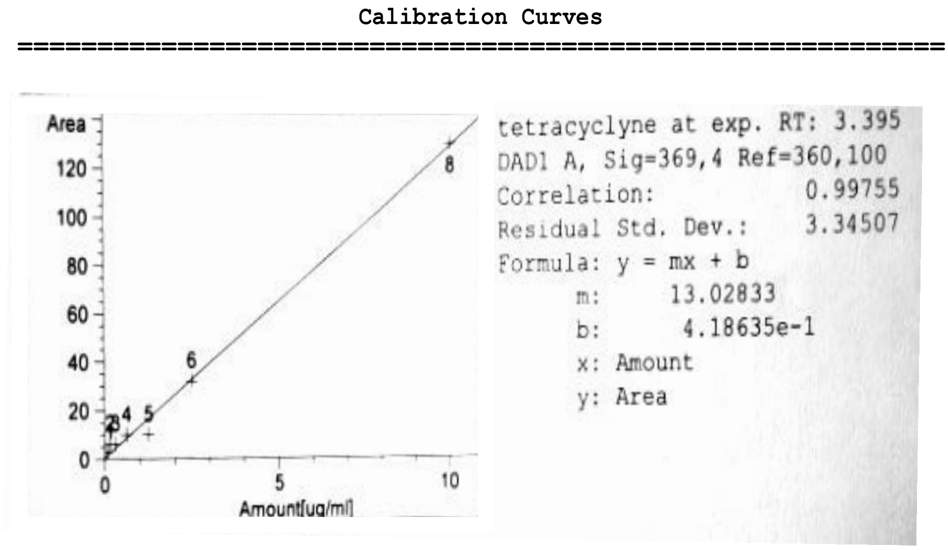

Figure 2. Standard calibration curve for tetracycline

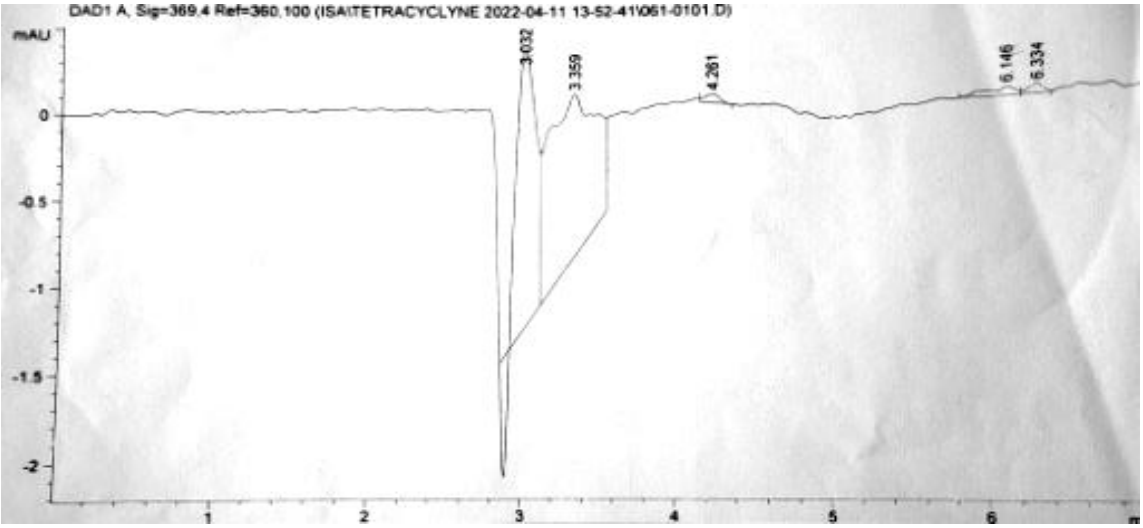

Figure 3. High-performance liquid chromatography chromatogram of tetracycline-positive milk sample. Note: TET: Tetracycline, Retention time: 3.359min

Discussion

This study revealed 23% (28/122) of local milk consumed in the study area contained antimicrobial residue. A higher prevalence of antimicrobial residue has been reported in other studies, such as a 25% prevalence reported by Yusuf et al. (2017). In similar studies in Kano City, Nigeria,40.8% and 62.3% of antimicrobial residue in raw and fermented milk were reported by Olatoye et al. (2016). Stella et al. (2020) also reported 76% and 85% antimicrobial residue prevalence in raw and fermented milk in Delta State, Nigeria. Another study reported 24% of antimicrobial residue in Bendar, Somalia (Mohamed et al. 2020). The 23% (28/122) antimicrobial residue prevalence reported in this study is higher compared to 0.69% of antibiotic residue in Croatia (Bilandzic et al. 2011) and 11-19% in Sweden (Shitandi and Sternesjo 2001). Differences in antimicrobial residue levels across reports and studies reveal differences in the degree of compliance with laws guiding antimicrobial usage by farmers and the implementation of existing laws guiding antimicrobial residue levels in food by regulatory agencies in the respective countries. Regulations such as withdrawal or withholding periods have been formulated for animal antimicrobials to protect the public from the health hazards associated with antimicrobial residues in foods of animal origin. Although there are laws against antimicrobials in food of animal origin in Nigeria, enforcement of such laws rarely occurs, especially with the prevailing traditional animal husbandry system. The findings of this study imply that consumers of local milk in the study area are exposed to a low risk of consuming one or more antimicrobial residues through the consumption of local milk. However, the results confirm non-compliance with the withdrawal periods by some local dairy farmers during the treatment of infections, as it is one of the most common ways antimicrobial residues are passed down to humans through the food chain.

Moreover, out of the 28 milk samples reported positive for antimicrobial residue in the screening test, 68% (19/28) were identified as tetracycline. That provides credence to the previous report, which mentioned tetracycline as one of Nigeria's most commonly used antibiotics, followed by penicillin, streptomycin, and sulfonamides (Alhaji et al. 2019). However, this study's overall prevalence of tetracycline was 15.6% (19/122). Additionally, the proportion of positive to negative tetracycline is significantly low ($P<0.05$), with probabilities of 0.16 and 0.84, respectively ($P<0.05$). From these findings, it can be inferred that local milk consumers in the study area are less likely to be exposed to tetracycline residue. In addition, the prevalence of residual tetracycline (15.6%) in this study is lower than that reported by Oluwafemi et al. (2018) (50%) in a similar study carried out in Abeokuta, Ogun State of Nigeria. The findings in this study were also lower than the prevalence in the Czech Republic (Navratilova et al. 2009), showing that all samples (100%) contained tetracycline, ranging from 5 to 24.47 µg/L. A study by Olatoye and Ehinmowo (2010) showed that tetracycline residue in meat samples in Akure, Nigeria had a higher prevalence of 54.44%, with the mean value of tetracycline residues in muscle, kidney, and liver were 51.8 µg/kg, 372.7 µg/kg and 1,197.7 µg/kg, respectively. Another study on seafood in Saudi Arabia by Alanazi et al. (2021) reported a 24% tetracycline prevalence, which is also above the findings of this present study. Although compliance with antimicrobial regulations in Nigeria is still low, the prevalence of tetracycline in this study is lower than in many previous reports. It can be due to antimicrobial residues other than tetracycline contaminating the food chain. Therefore, there is a reasonable level of compliance to antimicrobial regulations and responsible antimicrobial usage by most dairy farmers in the study area; there is a much higher use of traditional/alternative medicine or seasonal sample collection.

The concentration of tetracycline residue in this study ranged between 52.91 µg/L and 1,597.29 µg/L with a mean of 432.64 µg/L. This value is much higher than Navratilova et al. (2009) reported, which is between 5 and 24.47 µg/L.

It is also much higher than the tetracyclines concentration range in a previous study by Oluwafemi et al. (2018). Variations in the antibiotic levels in the present study can be attributed to the different health statuses of the dairy cattle from which the milk was collected, varying veterinary practices of the farmers, or milk processing (Oluwafemi et al. 2018). It is also interesting to report that the overall mean tetracycline residue level in the positive samples of this study was 432.64 µg/L which is much higher (four folds) than the maximum residue limits (MRLs) (100 µg/L in milk samples) recommended by the codex alimentarius commission maximum residue limits (MRLs). It suggests unprofessional administration of tetracycline in practice, which might have led to over or misuse. The presence of antimicrobial residue above MRLs in foods of animal origin is a global health hazard to human health (Stella et al. 2020).

In conclusion, this study revealed the occurrence of antimicrobial residue, including tetracycline, in the local milk consumed in the study region. That occurrence, though at a low prevalence but at dangerous levels. It could be related to the indiscriminate antimicrobial use in dairy cattle in the study area; therefore, the local milk consumers could be exposed to the potential hazard of tetracycline residue.

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REFERENCES

Aalipour F, Mirlohi M, Jalali M, Azadbakht L. 2015. Dietary exposure to tetracycline residues through milk consumption n Iran. J Environ Health Sci Eng 13: 80. DOI: 10.1186/s40201-015-0235-6.

Alanazi F, Almugbel R, Maheretal HM. 2021. Determination of tetracycline, oxytetracycline and chlortetracycline residues in seafood products of Saudi Arabia using high performance liquid chromatography–Photo diode array detection. Saudi Pharm J 29: 566-575. DOI: 10.1016/j.jsps.2021.04.017.

Alhaji NB, Aliyu MB, Ghali-Mohammed I, Odetokun IA. 2019. Survey on antimicrobial usage in local dairy cows in North-central Nigeria: Drivers for misuse and public health threats. PLoS ONE 14 (12): e0224949. DOI: 10.1371/journal. pone.0224949.

Bilandzic N, Kolanovic BS, Varenina I, Scortichini G, Annunziata L, Brstilo M, Rudand N. 2011. Veterinary drug residues determination in raw milk in Croatia. Food Control 22: 1941-19481. DOI: 10.1016/j.foodcont.2011.05.007.

Food and Agricultural Organization. 2008. Tolerances for residues of new animal drugs in foods. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=530.41

Kabrite S, Bou-Mitri C, El Hayek FJ, Hassan HF, Matar BJ. 2019. Identification and dietary exposure assessment of tetracycline and penicillin residues in fluid milk, yogurt, and labneh: A cross-sectional study in Lebanon. Vet World 12 (4): 527-534. DOI: 10.14202/vetworld.2019.527-534.

Kurjogi M, Issa MYH, Alghamdi S, Abdelrahman M, Satapute P, Jogaiah S. 2019. Detection and determination of stability of the antibiotic residues in cow's milk. PLoS ONE 14 (10): e0223475. DOI 10.1371/journal. pone.0223475.

Liu X, Steele JC, Meng XZ. 2017. Usage, residue and human health risk of antibiotics used in Chinese aquaculture: A review. Environ Pollut 233: 161-169. DOI: 10.1016/j.envpol.2017.01.003.

Marinou E, Samanidou VF, Papadoyannis IN. 2019. Development of a high pressure liquid chromatography with diode array detection method for the determination of four tetracycline residues in milk by using QuEChERS dispersive extraction. Separations 6 (2): 21. DOI: 10.3390/separations6020021.

Mohamed MA, Elmi AAS, Dubad AB, Hassan YHS, Osman AM, Bitrus AA. 2020. Antibiotic residue in raw milk collected from dairy farms and markets in Benadir, Somalia. PAMJ One Health 2 (19): 1-8. DOI 10.11604/pamj-oh.2020.2.19.24814.

Navratilova P, Borkovkova I, Drackova M, Janstova B, Vorlova L. 2009. Occurrence of tetracycline, chlortetracycline and oxytetracycline residues in raw cow's milk. Czech J Food Sci 27: 379-385. DOI: 10.17221/177/2008-CJFS.

Olatoye IO, Daniel OF and Ishola SA. 2016. Screening of antibiotics and chemical analysis of penicillin residue in fresh milk and traditional dairy products in Oyo State, Nigeria. Vet World 9 (9): 948-954. DOI: 10.14202/vetworld.2016.948-954.

Olatoye IO, Ehinmowo AA. 2010. Oxytetracycline residues in edible tissues of cattle slaughtered in Akure, Nigeria. Niger Vet J 31 (2): 93-102. DOI: 10.4314/nvj.v31i2.68952.

Olatoye IO, Ogundipe GAT. 2013. Quantitative analysis of oxytetracycline residue in beef and chicken meat from cities of southwest Nigeria. Bull Anim Health Prod Afr 61: 39-48.

Oluwafemi F, Oluwabamiwo BF, Oluwatosin OO, Akinrinade OR, Kolapo AL. 2018. Assessment of microbiological and residual antibiotics status in milk sold in Abeokuta, Ogun State. Nigeria. Turkish J Agric Food Sci Technol 6 (6): 642-651. DOI: 10.24925/turjaf.v6i6.642-651.1415.

Samandoulougou S, Ilboudo AJ, Bagre TS, Tapsoba FW, Savadogo A, Scippo ML, Traore AS. 2015. Screening of antibiotics residues in beef consumed in Ouagadougou, Burkina Faso. Afr J Food Sci 9 (6): 367-371. DOI: 10.5897/AJFS2015.1291.

Shitandi A, Sternesjo A. 2001. Detection of antimicrobial drug residues in Kenyan milk. J Food Saf 21 (4): 205-268. DOI: 10.1111/j.1745-4565.2001.tb00319.x.

Stella OO, Ezenduka EV, Anaelom N J. 2020. Screening for tylosin and other antimicrobial residues in fresh and fermented (nono) cow milk in Delta State, South-South, Nigeria. Vet World 13 (3): 458-464. DOI: 10.14202/vetworld.2020.458-464.

Van Boeckel TP, Brower C, Gilbert M, Grenfella BT, Levina SA, Robinson TP, Teillant A, Laxminarayan R. 2015. Global trends in antimicrobial use in food animals. Proc Natl Acad Sci USA 12 (18): 5649-5654. DOI: 10.1073/pnas.1503141112.

World Health Organization. 1999. Technical Report Series. Evaluation of certain veterinary drug residues in food (Fiftieth report of the Joint FAO/WHO Expert Committee on Food Additives). No. 888.

Yusuf MS, Kabir J, Bello M, Babashani M. 2017. Occurrence of tetracycline residues in raw milk from dairy farms in Kano State, Nigeria. Proc 54th Ann Congr Niger Vet Med Assoc Kano 2017: 155-164.