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Monitoring of Antibiotic Residues in Milk camels , cattle , goats and sheep in Elfasher Locality-North Darfur State

A. Salma , Y. H. Elmansory, E. B. Amna , E.S. Siham and M.A. Abdalla

A B S T R A C T

This study was conducted to detect the presence of antibiotics residues in camels , cattle , sheep and goats From North Darfur State . A total of 912 milk samples were collected From dairy Farms From camels 232 [25.4%] cattle 228 [25%] sheep 225 [24.7%] and goats 227 [24.9%] . during summer 300 [32.9%] , autumn 307 [33.7%] and winter 305 [33.4%] .All samples are screened using one plate test to detect the inhibition zone of bacillus sibitilues . The results revealed that 157 samples [17.2%] were positive for antibiotic residues , also in cattle the positive samples were 56 [6%] ,camels 46 [5%] , goats 34 [3.7%] and sheep 21 [2.3%] samples .The positive milk samples obtained in summer was higher [8.9%] than autumn [3.8%] and winter [4.6%] .The importance of this study is to develop monitoring system for controlling of antibiotic residues in cattle , camels , sheep and goats industries to save human health and to minimize drug resistance.

Keywords: antibiotic residues, caw, camels , sheepm, goats

INTERDCTON

Milk is a nutrient-rich, food-grade liquid, primarily secreted from the mammary glands of mature female mammals for the nourishment of young offsprings. In Africa, animal milk is from diverse female animals such as buffaloes, camels, cows, goats, and sheep. Cow milk accounts for a larger proportion (ca. 74%) of the total animal milk produced in the continent (FAOSTAT).

Milk contains many essential nutrients, so it is recommended to be consumed regularly by children. Consumers are looking for food that can improve their health. One kind of milk that can be used as food with good nutritional value is organic milk and has a higher selling value than milk derived from the conventional farming system because it implements high requirements regarding quality in the production and management process (Wanniatie , *2017*) .

Milk is an important and essential food for humane because of it is richness in nutrient s . It is highly valuable and consumed largely by people of all ages throughout the world . Milk is a perishable commodity susceptible for contamination with various chemical contaminants including anthelmintic drugs , chlorinated pesticides , organophosphates , herbicides , fungicides , antibiotics , hormones and disinfectants . Antibiotic contamination of milk poses significant threat to safety of milk and is regarded as global public health hazard . Antibiotics are widely used in dairy farming and milk production to inhibit growth of bacteria and promote the growth of dairy cows [Li *et al.*, 2019; Rana *et al.*, 2019] .

Worldwide meat consumption is on the rise , and according to the 2013 statistics from the United Nations Food and Agriculture Organisation (FAO 2021), the most consumed meat worldwide is pork , at 112 million tonnes annually , followed by poultry ,at 104 million tonnes annually. As a

means of coping with the rising demand for food, the use of antibiotics as growth promoters and feed enhancers were put into practice .In 2014, as high as 80% of the antibiotics produced and sold in the United States were used in
animals to promote growth and prevent infections. However, the uncontrolled application of these antibiotics at sub therapeutic doses in food animals, for purposes of growth promotion, has significantly contributed to the escalating antimicrobial resistance [Lekshmi *et al* ., 2017; Chantziaras *et al*., 2014; Elliott *et al* ., 2017] In 2017 , the United States Food and Administration (FDA) imposed new regulations to limit antibiotic use in livestock, where clinically important antibiotics are banned for purposes of growth promotion in animal husbandry [Brussow , 2017]. Although many of the world top meat-producing countries have banned the use of antibiotics as growth promoters in livestock countries such as China, Russia and India still allow farmers to use the antibiotic growth promoter in livestock [Van Boeckel *et al* ., 2015].
The presence of antibiotic residues in milk can be attributable to a number of different causes such as the misuse of antibiotics during treatment of lactating cows, disease prevention, failure to observe the withdrawal period and the illegal use of antibiotics as growth promoters [Fleming , 1929 ; Hotta *et al* ., 2018]
Antibiotic residues are the parent drug compounds and their metabolites excreted through edible tissues of animals to which antibiotic in question are administered . Antibiotic residues in milk are of major public health significance since it causes carcinogenicity , teratogenicity , drug allergy , bone marrow toxicity , mutagenicity and gastrointestinal disorders .
Carcinogenic effects have been found with antibiotics such as sulfa methazine , oxytetracycline , and furazolidone [Bacanli *et al* ., 2019]
Maximum residue limits of antibiotics regarding food help protect consumers but are no guarantees that animal products exceeding the limits will not come on to the market and be ultimately consumed by people . Even if the maximum residue limits for antibiotics in foods of animal origin are not exceeded they can still lead to problems in the long term , For example , antibiotics use in veterinary medicine were found in food , drinking water , and urine of preschoolers in Hong Kong [Li , *et al.*, 2017] .
It is necessary to monitor the presence of antimicrobial drug residues from not entering the human food supply [Moudgilp, 2019 ; Warsma *et al*., 2020 ; Addoma *et al*., 2016 ; Kirrolia and Nehra 2012 ; Navratilova 2008 ; Kressc *et al* ., 2007] Currently used detection methods are mostly based on chromatographic, immunological and microbiological test procedures[Sachi *et al* 2019 ; Shen *et al* 2019 ; Kloth *et al* 2009 ; Mukunzi *et al* ., 2017]. Nowadays , immunological tests such as ELISA or lateral flow assays (LFAS) as well as microbial test kits are commonly used for monitoring of milk samples [Sachi et al 2019 ; Shen *et al* 2019 ; Mukunzi *et al* ., 2017].

MATERIAL AND METHOD

Sample Collection

A total of 912 milk samples were collected randomly from dairy farm and central market during summer 300[32.9%] , autumn 307 [33.7] and winter 305 [33.4%] from camels 232 [25.4%] , cattle 228[25%] , sheep 225 [24.7%] and goats 227 [24.9%] between December 2017 to December 2018.
The milk samples were put in sterile container stored at 20c .Ice boxes were used to carry the samples from the collection centers to Laboratory and transferred to the Central Veterinary Research Laboratory under refrigerated condition and stored in deep freezer at-20 C until analysis
.

Cultivation Media and Solutions

One plat Screeing test method was used simultaneously for the determination of the presence antibiotics residues in milk samples that included microbiological inhibition assay.

Physiological or isotonic saline was prepared by dissolving 0.085g of sodium chloride in 100ml of distilled water then sterilized by autoclaving at 121 C for 15minutes.

Twenty eight grams of nutrient agar medium(Oxoid , 2006) was suspended in one liter distilled water, dissolved completely by boiling in water bath for one minute then autoclaving at 121 C for 15mintues then it was cooled . After that it was allowed to cool at room temperature at 25 c . Finally it was stored in the incubator over night to dry .

Bacillus subtilis (ATCC 6633) was obtained from the Department of Bacteriology, Central Veterinary Research laboratory, Khartoum , Sudan and used as an indicator organism according to the method described by Ellerbroek [Ellerbroek L. 1991] .This bacteria is highly sensitive for multiple antibiotic including pencillin and tetracycline. Muller Hinton media it is a selective media for Bacillus subtilis thus inhibiting growth of other , with some modifications. Under flame a sterile loop was used to transfer some colonies of *Bacillus subtilis* previously grown on nutrient agar slant, on to a nutrient agar plate and incubated at 37c for 18 hours . One colony from the fresh culture was taken by using the sterile loop and diluted in 9 ml sterile 0.85% NaCl. vortex mixer mixed the suspension then adjusted to 0.5 in a McFarland Standard turbidity tube (equivalent to 3*10 cell/ml).

Microbial Inhibition assay (MIA)

All samples were screened for the presence of antibiotic residues by microbial inhibition assay utilizing *Bacillus subtilis* as test organism according to the method described by (Dinki and Balcha, 2013) with some modification. The plates containing 25 ml of nutrient agar were inoculated with *B. subtilis* by swabbing the entire agar surface using sterile cotton swabs dipped in suspension of the organism. Sterile discs (filter paper procedure consist of 125mm diameter made with the help of punch machine) were dipped in milk samples and excess milk was removed by pressing gently against sides of tubes with the aid of a long narrow forcep and then Six discs were placed in each plate at equal distance from each other. The plates were incubated at 37 C for 18 to 24 h. The samples producing clear zone of inhibition around the discs were considered positive for antibiotic residues.

Result and Discussion

As shown in Table (1) all Samples were detected by microbial inhibition one plate test and the Positive to antibiotic were 157 (17.2%) and the negative were 755 (82.7%) .

Cattle milk revealed high percentage (6.1%) than camels (5%) , sheep(2.3%) and goats (3.7%)

Also in this result the number of positive sample to antibiotics were (8.9%) in summer high percentage than autumn (3.8%) and winter (4.5%) .

From this result the usage of antibiotic was increased in hot seasons than cold seasons .

In Sudan there is little data concerning the presence of antibiotic resides in the food sold for human Consumption . The present study using one plate test for monitoring antibiotic residue revealed considerable percentage in milk cattle , camels , goats and sheep collected from dairy farm and central market in El fashir Locality.

The sample is consider positive if only the zone of inhibition > 2 mm occur at least around sample (6-11mm diameter) .

The higher result in cattle rather than camels , goats and sheep due to cattle milk sold at the markets for human consumption and the most consumed milk for human use .

People in African use milk from cows , sheep , goats , and camels , as source , cows milk it the most widely produced and processed (FAO , 1990) .
The significant difference in the prevalence of antibiotics in milk in the different seasons of the year occurred due to the different diseases and medicines used . Samples of collected milk in summer showed high percentage to those which were collected in autumn and winter that in dicates effects of higher temperature on bacterial growth . Bacteria growth and reproduction are affected by the seasons . Samples of collected milk in winter shows higher percentage than autumn due to in winter when weather becomes cold diseases such as pneumonia increased and farmers used antibiotics to treat animals and antibiotics residue transferred in to milk .

Table.1 Percentage of milk Cattle , Camels, goats and sheep (n= 912) in Elfashir Locality – North Darfur State

Samples	Frequency	Percent
Positive	157	17.21
Negative	755	82.7
Total	912	100

Many previous surveys were employed for detection of residual antibiotics in the milk the Khartoum State (Omer , 2016) applied delvotest SP for detection of antibiotics residues in 236 milk samples. He obtained about 21.18% positive results claimed that which is nearly closed to our study. Barakat (1995) detected antibiotics residues in 80 milk samples by using delvotest P . He an nounced that 8.75% which is lower than preset results. Osman (2002) claimed that the percentage of positive samples for total samples examined was 0.8% and for the samples taken directly from the udder it was 4.0% , while Mustafa (2002) searched for the antibiotics resides in 100 milk samples collected from different areas in Khartoum State and his results proved negative for all the samples investigated . Said Ahmed *et al* , (2008) reported 17% of milk samples were positive in Khartoum and 11% Khartoum North ,this result was similar to our study
In Zimbabwe 73 samples of raw milk from 3 main dairy market board collection center s were scanned for the presence of microbial growth inhibitory substances and 4.4% of the samples contained antibiotic residues (Chagonda and Ndiku 1989) , this result is more lower to the results obtained in this study . In Lisbon 2248 samples of consumer milk were examined in 1981 to 1985 . Six hundred and seventy four of them (30%) contained inhibitory substances (Barbosa *et al*, 1991) .this result is higher than our study.
These differences might be due to the effect of seasons or type of test used.
In Sudan more than 80% of veterinarians did not determine animal weight when describing doses, no following up of cases after leaving the clinic , slaughtered animals and milk during treatment without completing withdrawal periods (Mohammed *et al*., 2011) .
In milk industries the producers used a variety of products to control diseases and increase yields . the risks of residues in foodstuff from animal origin could be reflected into several forms , carcinogens , allergies toxicity alteration of the intestinal flora, bacteria resistant (Wageh *et al*., 2013;Mohamed *et al*., 2019). The risk of violative drug residues is minimized when treatment protocols are carefully followed.
In conclusion, the antibiotic abuse is most important cause of high prevalence of residue and large number of resistant bacteria. Developing monitoring system for screening residues in food is an important issue now a day to produce food and to meet the international standard to enhance international trade.

In EL fashir many factors affect the presence of antibiotics residues in milk such as mal practice of milk venders who add antibiotics to milk to avoid the effect of bacteria , when there are delays in milk marketing . Also the milkers don't comply with the many antibiotics withdrawal time when they treat their animals as some any milk these animals in the same day of treatment .

This study suggested that more efforts are needed to enhance and promote farms and markets (sale points) of milk by developing screening confirmatory tests on sale points and farm milk .Moreover , the ministries concerned should adopt comprehensive strategy for ensuring a safe milk supply of good quality . these strategies should include promoting knowledge of farmers standard by teaching and extension and the adoption of grading quality testing of milk . Ultimately, the milk testing programs should become component of the quality process that is focus on the milk farms and the producers in producing high quality milk .

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