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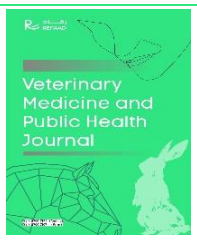

Chemical Preservatives, Adulterants, and Antibiotic Residues in Raw Cow's Milk in Khartoum State, Sudan

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Abstract:

Objective: This study investigate the influence of season on the occurrence of adulteration by chemical preservatives and antibiotic residues (beta-lactam, tetracycline and sulfonamides) in the raw milk in the main towns of Khartoum State, Sudan.

Methods: The milk samples (n= 270) were randomly collected from farms (90 samples), groceries (90 samples) and vendors (90 samples) during late summer and winter seasons, 135 samples during each season. The samples were placed in cool cracked ice and transported immediately to the laboratory for analysis.

Results: Data revealed a high presence of the added water in the milk collected from vendors during late summer season (7.70± 9.46%). Also, hydrogen peroxide, starch and formalin were detected only in the milk obtained from vendors. However, the higher percentages of salt (32%) and ammonium sulphate (28%) were obtained in the milk samples collected from the groceries during late summer season, while the occurrenc of ammonium sulphate was 26% in the samples collected from vendors during winter season. On the other hand, 93% of the milk samples examined during late summer season were positive for the occurrence of antibiotic residues compared to 55% that was detected during winter season. The highest percentage of antibiotic residues (26%) was found in milk from groceries during the winter season. Milk samples from vendors during late summer season contained beta-lactam, tetracycline, and sulfonamide in 19%, 13%, and 1% of samples, respectively, while these figures were 20%, 2%, and 3%, respectively, during winter season.

Conclusions: The addition of water followed by the presence of salt and antibiotics are the most adulterants found in raw milk sold in Khartoum State especially from vendors during late summer season.

Keywords: *milk adulteration; antibiotic residues; chemical preservatives; seasons.*

1 Introduction

Milk and dairy foods are valuable in supplying a wide range of key nutrients, of which some are particularly vital for certain stages of life (Givens, 2020). The increased consumption of milk and its products is a result of the research findings that milk is rich in nutrients that are important for the human diet (Lambrini et al., 2021). Moreover, production and consumption of milk and its products have been increasing over the past 30 years globally due to their nutritional benefits (Montgomery et al., 2021). Furthermore, for the food industry, dairy products are important due to expected increase in future consumption. However, faster and precise authentication of these products is urgently required to safeguard the public health (Hebling e Tavares et al., 2022).

The increased demand and complexity of the milk supply chain, in addition to competition in the dairy market, have led some unscrupulous producers to practice adulteration of milk in developing countries, where strict vigilance by food safety authorities is lacking (Handford et al., 2015). Financial gain is the major reason for milk fraud, when there is inadequate supply to satisfy and to meet the demand of the growing population in the world occurred. This problem is particularly acute in the developing and underdeveloped countries that lack the adequate laws and monitoring (Azad and Ahmed, 2016). Moreover, Afzal et al. (2011) reported that the middlemen do not care to maintain proper hygiene when transporting the milk in addition to their practice of adulterating the milk using starch, flour, sugar cane, vegetable oils, urea, detergents and antibiotics to increase the shelf life of milk in order to increase their margin profit. Water is the commonly used adulteranst because it is cheap, its addition to the milk increases its volume and, hence decreasing its nutritional content. Additionally, milk producers add preservatives like caustic soda to reduce milk acidity to increase its shelf life, as well as milk powder, starch, sugar, and urea to increase its solids-not-fat content (Dangi, 2021).

The distribution of milk in Sudan is through irregular marketing channels by vendors using donkeys or cars, collection centers and directly from the farms. These informal and uncontrollable channels resulted in reduction of the compositional values of milk by subjecting it to adulteration (Elsheikh et al., 2015; Mohammed and El Zubeir, 2021; Abdalmahmoud et al., 2023). Moreover, some of the producers and vendors are found to add illegal preservatives and neutralizers for extending the shelf life and the perishability of milk (Mohammed and El Zubeir, 2021). Additionally, residues of beta-lactam antibiotics were found in different animal products particularly in milk from treated cows (Gritli et al., 2021). Thus, monitoring is necessary to prevent antimicrobial drug residues from entering the human food supply (Addoma et al., 2016; Warsma et al., 2021). The main objective of this study is to investigate milk adulteration in different sources (farms, groceries and vendors) in Khartoum State during the late summer and winter seasons. It is also meant to identify the different types of chemical (formalin, hydrogen peroxide, ammonium sulphate and salt) and antibiotic residues (beta-lactam, tetracycline and sulfonamide) added to preserve the milk.

2 Materials and Methods

2.1 Sources of milk samples

The raw milk examined in this study was collected from the 3 main cities of Khartoum State (Khartoum, Khartoum North and Omdurman); 135 samples during June to July 2016 for late summer and 135 samples during October- December 2016 for winter. Bulk homogenized milk samples were collected randomly after the morning or evening milking from the investigated farms or purchased from groceries and vendors distributing their milk for sale in Khartoum (90 samples), Khartoum North (90 samples) and Omdurman (90 samples). The samples examined were 45 during late summer and winter season from each city.

The milk collected include 15 samples from groceries, farms and vendors in each city. The aim was to detect the milk adulteration with chemical preservatives (formalin, starch, boric acid, ammonium sulfate, hydrogen peroxide, added water) and the presence of antibiotics residues (beta-lactams, sulfonamides and tetracycline).

2.2 Tests for detection of chemical preservatives and antibiotic residues

The addition of water was examined using milk Lactoscan analyzer (Milkotronic LTD, Nova Zagora, Bulgaria). Tests for detection of starch was determined according to Kumar et al. (1998). Meanwhile, formaldehyde, salt and ammonium sulphate were estimated as outlined by Foley et al. (1974). The occurrence of hydrogen peroxide was conducted according to Recio et al. (2000). The antibiotic residues were detected by using tri-sensor test (Unisensor

Company, Liege, Belgium). using the methods described by the manufacturer's instructions. Trisensor kits are easy to use, fast and efficient in combining 3 or more antibiotics screening test for their residues simultaneously in the milk (Warsma et al., 2020).

2.3 Statistical analysis

The obtained data were statistically analyzed using SPSS program version 16 (SPSS, 2008). The ANOVA test was conducted for the generated data and General Linear Models were used to determine the percentage of the added water in the examined milk samples. Means were separated using Duncan Multiple Range Test ($P \leq 0.05$). The figures were plotted using excel Microsoft sheet.

3 Results

3.1 Adulteration of milk with added water

The average added water of milk samples collected during late summer season (7.70± 1.46%) revealed significant ($P < 0.001$) high values compared to the milk samples examined during winter season (5.05± 1.45%). The means of added water (Table 1) of milk samples collected from Khartoum North (7.78± 1.45%) showed higher value in comparison to those obtained from Khartoum (6.13± 1.55%) and Omdurman (5.36± 1.99%). The differences in added water of milk samples collected from Khartoum, Khartoum North and Omdurman cities were not significant ($P > 0.05$). However, milk samples were significantly ($P < 0.01$) affected by the source from which the milk samples were taken (Table 1). As the average water added to the milk samples obtained from the farms (8.25± 0.56%) was significantly ($P < 0.01$) higher than those purchased from the groceries (2.29± 0.98%).

Table 1: Presence of added water in milk samples collected from different sources in Khartoum State during late summer and winter seasons

Seasons		Significant level	Location			Significant level	Source			Significant level
Late summer	Winter		Khartoum	Khartoum North	Omdurman		Farms	Groceries	Vendors	
$7.70^{a} \pm 1.46$	$5.05^{b} \pm 1.45$	***	$6.13^{a} \pm 1.55$	$7.78^{a} \pm 1.45$	$5.36^{a} \pm 1.99$	NS	$8.25^{a} \pm 0.56$	$2.29^{b} \pm 0.98$	$5.25^{b} \pm 0.24$	**

3.2 Presence of salt, starch, formalin and hydrogen peroxide in milk samples collected from different sources during late summer and winter seasons

Fig. 1 indicated that the samples of milk collected from farms and groceries during late summer and winter seasons showed negative result for starch, hydrogen peroxide and formalin. However, 4% and 3% of the samples taken from vendors during late summer season were positive for the presence of hydrogen peroxide and formalin, respectively. Meanwhile, 3% of samples obtained from the vendors during winter season were positive for starch.

Data in Fig. 1 showed that 73% of the milk samples examined during late summer season were

positive for salt; 32%, 21% and 20% in the milk samples taken from groceries, vendors and farms, respectively (Fig. 1). However, 67% of the milk samples examined during winter season revealed the presence of salt in the milk samples collected from groceries, vendors and farms; in 28%, 28% and 11%, respectively (Fig. 1).

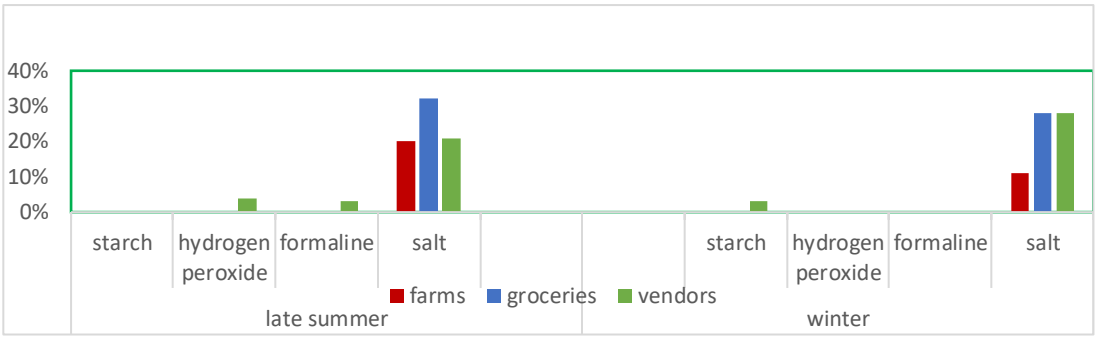

Fig. 1: Presence of salt, starch, formalin and hydrogen peroxide in milk samples collected from different sources during late summer and winter seasons

3.3 Presence of ammonium sulphate in milk samples collected during late summer and winter seasons from different sources in Khartoum State

The present result (Fig. 2) indicated that 64% of the examined milk samples collected during late summer season were positive for the occurrence of ammonium sulphate, of which 28% were found in the milk obtained from groceries, 20% in the samples taken from farms and 16% in the samples collected from vendors. Also, 65% of the milk samples examined during winter season (27%, 26% and 12% from vendors, farms and groceries) were found positive for the presence of ammonium sulphate (Fig. 2).

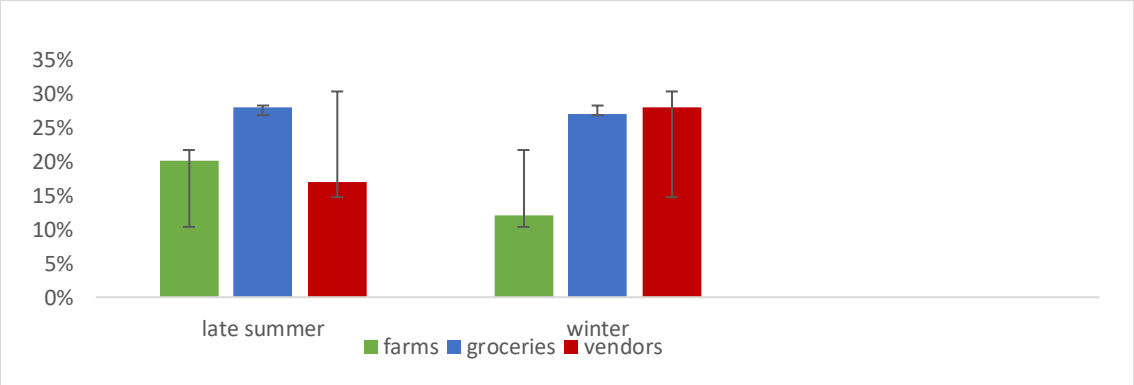

Fig. 2: Presence of ammonium sulphate in milk samples collected during late summer and winter seasons from different sources in Khartoum State

3.4 Presence of antibiotics in milk samples collected during late summer and winter seasons from different sources in Khartoum State

Data in Fig. 3 showed that 23% and 8% of the milk samples collected from farms during late summer season were positive for the presence of beta-lactam and tetracycline, respectively. However, all the examined milk samples from farms were free from sulfonamide during late summer season. Also, 18% of the samples purchased from the groceries were found positive for beta-lactam, 10% were positive for tetracycline and 1% was positive for sulfonamide. Moreover, 19% of the milk samples obtained from vendors during late summer season were positive for beta-lactam, 13% were positive for tetracycline and 1% was positive for sulfonamide (Fig. 3).

Fig. 3 illustrated that 39% of the collected milk samples from farms during winter season were positive for antibiotics; 5% were positive for beta-lactam, 14% were positive for tetracycline and 20%

were positive for sulfonamide. However, the milk samples collected from the groceries during winter season showed the occurrence of beta-lactam in 5% of the examined samples and 2% of the positive samples for each tetracycline and sulfonamide residues (Fig. 3). Also, the milk samples purchased from vendors showed that 25% of the milk samples were positive for antibiotics; 20% positive for beta-lactam, 2% positive for tetracycline and 3% positive for sulfonamide (Fig. 3).

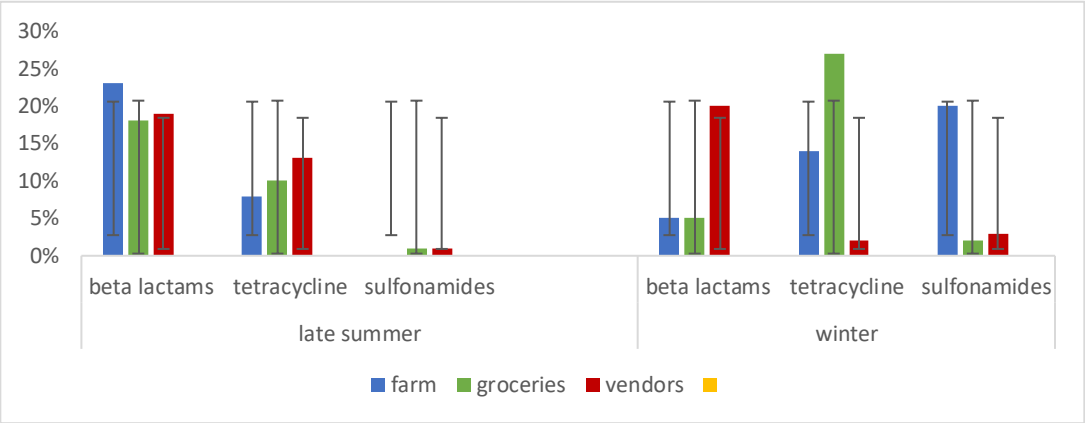

Fig. 3: Presence of antibiotics in milk samples collected during late summer and winter seasons from different sources in Khartoum State

3.5. Presence of antibiotics in milk samples collected during late summer and winter seasons from the towns of Khartoum State

All the examined milk samples collected from the different sources in Khartoum city during late summer season were free from sulfonamide (Fig. 4). However, 22% of the milk samples showed positive occurrence of beta-lactam and 7% were positive for tetracycline. The samples collected from Khartoum city during winter season revealed the occurrence of 14% positive beta-lactam in the examined milk samples, 13% were positive for tetracycline and 3% were positive for sulfonamide. Meanwhile, data in Fig. 4 showed that 35% of the examined milk samples from Khartoum North during late summer season were positive for antibiotics, of which 20% showed beta-lactam, 14% containing tetracycline and 1% was positive for sulfonamide. However, 12% of the milk samples examined were positive for beta-lactam, while, 3% were positive for tetracycline and 1% was positive for sulfonamide during winter season (Fig. 4). Meanwhile, 27% of the antibiotics positive milk samples collected from Omdurman during late summer season showed frequencies of 14%, 11% and 2% for beta-lactam, tetracycline and sulfonamide, respectively. However, the milk samples obtained from Omdurman city during winter season were free from sulfonamide, while 12% were positive for the presence of beta-lactam and 3% for tetracycline (Fig. 4).

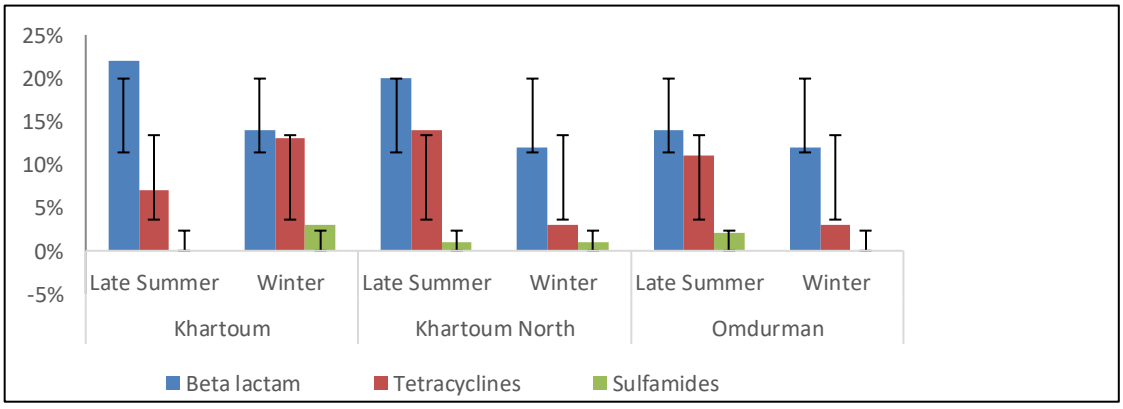

Fig. 4: Presence of antibiotics in milk samples collected during late summer and winter seasons from Khartoum State

4 Discussion

It is clear from the presented results that adulteration of milk samples examined in Khartoum State with added water was not affected by the location (Table 1). However, it showed highly significant differences during late summer and winter season. The milk samples examined during late summer season showed high added water compared to those tested during winter season. Water is added most commonly as a practice of adulterating the milk to increase its volume and reduces its nutritional value (Dangi, 2021). The adulteration practice by adding ice to milk in order to maintain the shelf life during late summer season was reported (Chanda et al., 2012). Similarly, Memon et al. (2018) reported that water was found as a common adulterants in all the examined milk samples collected from different sources in Hyderabad, India. Similarly, all the milk samples collected from Bangladesh were adulterated by water (Chanda et al., 2012). However, Mohammed and El Zubeir (2021) found 16% of the examined milk samples collected from Omdurman, Sudan contained added water.

Addition of water to the milk was significantly affected by the sources of milk (Table 1), it was high in farms. Also, high percent of the added water was found for the samples taken from the farms (10.6± 11.6%) and sale points (10.6± 10.4%) compared to those from the groceries (8.7± 8.2%) in Gedarif town, Sudan (Abdalmahmoud et al., 2023). Kandapal et al. (2012) reported that 80% of the milk samples obtained from different sources were adulterated with water. Moreover, Barham et al. (2018) found that added water was found remarkably ($P < 0.05$) high (83%) than other adulterants. In addition, adulteration of milk with water was about 97% in the canteens of educational institutes and 93% in the canteens of the public places in Faisalabad, Pakistan (Lateef et al., 2009). Hebling e Tavares et al. (2022) reported that water is primarily used to increase the milk volume and as the content of fat and protein decrease by the added water other forms of adulteration were practiced in order to mask their level of reduction. Lower contents of solids not fat, fat, protein and density were shown for the cow's milk with added water (Abdalmahmoud et al., 2023). When milk is diluted with water, their nutritional values were reduce and if contaminated water was added, additional health problems are most commonly occur (Dangi, 2021).

The presence of starch was found in the milk purchased from vendors (Figure 1). This result agreed with those obtained by Arora et al. (2004). Also, Ahmad (2009) reported that 35.5% of milk samples collected from different localities in Khartoum North were positive for starch. Meanwhile, Muntaha et al. (2020) found that 33% of milk samples were adulterated with starch in Faisalabad. However, Mohammed and El Zubeir (2021) reported that all milk samples collected from Omdurman, Sudan showed no starch.

Hydrogen peroxide was detected in the milk samples purchased from vendors during late summer season (Figure 1). This result was agreed with those found by Costa et al. (2020). Also, Ongarora and Karwimbo (2019) reported that 70% of the milk samples collected from Nairobi were adulterated with hydrogen peroxide. However, Ananya et al. (2015) found the presence of hydrogen peroxide in 9.68% of the examined milk samples. The current results were in disagreemeant to those obtained by Mohammed and El Zubeir (2021) who reported that hydrogen peroxide was not found in all milk samples collected from Omdurman, Sudan.

Formalin was detected in milk samples collected from vendors during late summer season (Figure 1). Similarly, Mohammed and El Zubeir (2021) found that 10% of milk samples transported using cars and 13% transported using donkeys in South Omdurman were contaminated by formalin. Also, high contamination of the milk samples by formalin wes found in the sale points compared to the farms (El Zubeir and El Owni, 2009). The reason as they suggested might be because some of the milk sellers add chemical preservatives to the milk to prolong its shelf life. Moreover, Karmaker et al.

(2020) reported that when formalin was added at a rate of 0.01 and 0.4% on cow milk that stored at refrigeration temperature for up to 3 days, the milk showed no changes in appearance of formalin. However, Silva et al. (2015) evaluated the presence of formalin residues during refrigeration and found that formalin was detected after 48 hours after refrigeration. The results of this study disagreed with those obtained by Shinawy et al. (2018) who indicated that all examined milk samples were free from formalin residues. Milk adulteration using preservatives including formalin resulted in serious health hazards like the damage of liver and kidney due to its toxic effect. Among the harmful effects are increasing of asthma problem and hyper activities in children (Dangi, 2021).

Salt was found in all milk samples obtained from different sources in different locations except the farms located in Khartoum city during late summer season (Figure 1). Similarly, Nirwal et al. (2013) mentioned that 51% of the milk samples collected from different localities in India were adulterated with salt. Also, Chugh and Kaur (2022) found that salt was the major adulterants used for milk in Kadapa and Andhra-Pradish in India. The result of this study agreed with those reported by Barham et al. (2015) who found that from 100 milk samples, 17% of the samples were adulterated with salt. The result of this study disagreed with those found by Al Mamun et al. (2016) who found that in all 25 milk samples examined no positive samples was found for salt test. However, Rao et al. (2018) found that salt was the most common adulterants, 45% of studied milk samples were adulterated with salt in Hyderabad. This result was similar to Lai et al. (2020) who found that 60.71% of milk samples were detected positive for salt test. Usually, salt is added to the milk mainly to increase and to correct the reading of the Lactometer to increase the density value of the diluted milk or to increase the pH value (Rao et al., 2018). Carbonates and bi-carbonates are usually added to neutralized the pH and acidity of the badly preserved milk and to pass it as fresh milk. However, carbonates and bi-carbonates can cause disruption in hormone and affecting the regular development and reproduction (Dangi, 2021).

The presence of ammonium sulphate was found higher in the milk samples examined during late summer season (Figure 2) and the high percentage was found in the samples purchased from the groceries in Khartoum North city. Similarly, Roy et al. (2017) found that the percentage of ammonium sulphate in the samples collected from east Delhi was higher than the samples collected from Faridabad and that all milk samples collected were containing ammonium sulphate. Also, Raju et al. (2017) reported that of the examined milk samples, 37% were found to be adulterated with ammonium sulphate. Moreover, Makadiya and Pandey (2015) reported that from the 30 examined milk samples, 29 were adulterated with ammonium sulphate. However, Shinawy et al. (2018) and Suradakar et al. (2015) found that all studied milk samples were free of ammonium sulphate. Generally, ammonium sulphate is added to milk to raise its density when the milk diluted with water (Roy et al., 2017).

In the present study, the higher occurrence of antibiotics was found in the milk samples collected from vendors (Figure 3). This might be due to the improper treatment of the diseases, lack of pasteurization and cooling facilities, variation in the management practice and the level of awareness among the workers and how to deal with animals being treated with antibiotics (Mohamed and El Zubeir, 2007). According to Addoma et al. (2016), 73.3% of the veterinarians in Nyala believe that it is not necessary to prescribe the drug and that 43.3% of them practice wholesale of antibiotics to dairy farm owners. Moreover, 93.3% of the interviewed veterinarians stated that most of the saleable drugs are broad spectrum antibiotics. This abuse and misuse of antibiotic is one of important risk factors in causing high rate of drugs residues and hence increasing the antimicrobial resistant of the bacteria (El Zuber et al., 2012).

The antibiotic residues were found high in the milk samples collected from farms during late

summer season (Figure 3). Previously, the milk taken from cows in Khartoum State showed that 40% of the samples were positive to antibiotics residues test (Warsma et al., 2021). However, Ondieki et al. (2017) found that 32 (15.5%) and 28 (18.4%) of the milk samples obtained from farmers and vendors respectively, were positive for antimicrobial residues. About 80% of dairy herds were found to relay on antibiotic therapy to treat animals against infections including mastitis, the residues of these antibiotics are deposited and present within the milk (Gustavsson et al., 2002). Generally, the commonlly used antimicrobial medications are tetracyclines, sulfonamide and nitrofurans (Alomirah et al., 2007). Beta-lactam drugs like penicillin-G, amoxicillin, cloxacillin, dicloxacillin, cefuroxime and cefoperazone are also used (Sierra et al., 2009). The safe milk should be free from antibiotic residues (Said Ahmed et al., 2008). This because consuming of contaminated animal products with antibiotic residues might cause allergic reactions and reduce the efficacy of antibiotics for treatment of infections in human (Warsma et al., 2021). Although, pasteurization and other methods of temperature treatment are effective for pathogens, they inefficient for eliminating drug residues (Tang et al., 2009). Addoma et al. (2016) found the contamination rate with tetracycline was in 34% and 31% in the raw and heated milk samples respectively, while sulfonamides was found in 31% and 29% in the raw and heated milk samples, respectively.

The milk samples tested positive to the antibiotics were high in milk samples examined during late summer (91%) than the samples collected during winter (61%). beta-lactams were the major detected residues (Figure 4), Similarly, Hajmohammadi et al. (2021) reported that penicillin residues were high in the milk samples examined both during summer (100%) and winter (97%). Moreover, Wanjala et al. (2018) found that 21.1% of the studied milk samples had residues of antimicrobial. This result was similar to those found by Warsma et al. (2020) who reported that 40% of cow milk samples collected from Khartoum State were positive for antibiotic residues; 12% and 28% positive samples were found during winter and summer, respectively. However, Said Ahmed et al. (2008) found that the cow milk samples showing positive reaction for the antibiotic residues were 28%. Moreover, Addoma et al. (2016) found the occurrence of beta-lactam was high in milk samples obtained from the sale points (37%) compared to those collected from dairy farms (32%) in Nyala, South Darfur State, Sudan. Hence, vaccination programs for dairy cows targeting the epidemic diseases should be applied in order to reduce treatment using antibiotics, in addition to organizing an educational program on the proper uses of antibiotics and their withdrawal period among the farms owners (Warsma et al., 2021). In Sudan, most of the dairy farmers always decide which drugs to use depending to their own experience or their neighbors farmers, which could be due to the easiness in obtaining veterinary medicinal products (El Zubeir and Mahala, 2011). Moreover, nearly half of the veterinarians (56.7%) advise the dairy farmers regarging the importance of drug withdrawal period (Addoma et al., 2016). Also, the One Health approach should be considered when using antibiotics for production animals (Gritli et al., 2021). The supervision of the dairy farms by the veterinarian was found to reduce the risk factors associated production, reproduction, occurrence of diseases, lack of washing hands of laborers before milking and. eliminating the presence of other animals in the cows' pen (Yousif and El Zubeir, 2024).

The presence of beta-lactam was higher compared to tetracycline and sulfonamide (Figure 4). However, Rahman et al. (2020) reported that milk samples were recognized as positive against all 5 tested antibiotics that include amoxicillin, oxytetracycline, streptomycin, gentamicin, and ceftriaxone. The occurrence of beta-lactam residues in milk is a real threat to the health of both animals and human, thus in order to reduce these risks a systematic monitoring should be implemented for milk production (Gritli et al., 2021).

The obtained result is not supporting the findings of Serraino et al. (2016), they found that 64 samples collected from milk trucks showed antimicrobial residues levels, which are exceeding the detection limit and that sulfonamide residues revealed 3.4% compared to tetracycline (0.3%), penicillins (0.03%) and cephalosporins (0.03%). However, Mohamed et al. (2020) found that 30% of the milk samples collected from farms contained antibiotic residues compared to 18% of the milk samples collected from the markets. Thus the farmers, veterinarians and the dairy industry; as the main stakeholders in the milk industry; has important roles in reducing the presence of antibiotic residues in milk (Gritli et al., 2021). Hence the risk factors associated with drugs residues in milk including the veterinarian practices, the unawareness of dairy farms' owners and their mismanagement should be addressed via legislation, monitoring and training programs to avoid the risk that might arise (Addoma et al., 2016). Initiation of proper milk collection is needed to reduce the practice of adulterating to ensure the consumption of good quality milk without any risk of health hazard to humans (Mohammed and El Zubeir, 2021). Training of farm owners and workers to manage and improve the health level in the dairy farm, its biosecurity measurement and disease control are highly required to improve dairy production in the country (Yousif and El Zubeir, 2024).

5 Conclusion

From the present findings, it can be concluded that the quality of raw milk sold in Khartoum State is low especially the milk sold by vendors during the late summer. The added water and the presence of salt and antibiotics are common adulterants in raw milk. These results suggest that most milk vendors are adulterating the milk to prolong its shelf life and to increase profits. Hence, implementation of a reliable and efficient quality control system to monitor the milk supply chain regularly by the official regulatory authorities should be maintained. Additionally, the establishment of proper milk collection and marketing systems should be the target to avoid the risks of milk adulteration and contamination.

5.1 Acknowledgments

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