10.21608/avmj.2024.318055.1384 Assiut University website: www.aun.edu.eg

RISK ASSESSMENT OF OXYTETRACYCLINE AND PENICILLIN RESIDUES IN RAW MILK

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Received: 1 September 2024; **Accepted:** 3 October 2024

ABSTRACT

Antibiotic residues in milk are of great public health concern since milk is widely consumed by infants, youngest and adults throughout the globe. The present work aimed to determine the presence and concentration of the residues of oxytetracycline and penicillin in raw milk samples. A total of 120 random raw milk samples were collected from different dairy shops, through summer and winter, dairy farms and farmers' houses (30 samples for each) in Assiut City, Egypt. A double-beam UV/visible spectrophotometer was used to detect and determine the residues. The incidences of oxytetracycline residues in summer dairy shops s, winter dairy shops s, and farm and farmers' houses milk were 21, 25, 29 and 28 %, respectively. The incidences of penicillin residues in the same samples were 28, 26, 25 and 27 %, respectively. The minimum and maximum for oxytetracycline were 3 and 38.65 µg/L, respectively with an average of 8.99 µg/L, while for penicillin were 5.9 and 250 µg/L, respectively, with an average of 76.5 µg/L. All detected oxytetracycline residues were below the 100 µg/L MRL standards, while all the penicillin residues were above the 4 µg/L MRL standards set by Codex. Of the total samples, 6 samples were free from residues, 18 samples had one antibiotic residue, and 96 samples had two antibiotic residues. A comparison was made between the permissible daily consumption and the calculated daily intake for each antibiotic residue. A Hazard Quotient was carried out for risk evaluation of the residues of penicillin and oxytetracycline in adults and children.

Keywords: Antibiotic residues, Oxytetracycline, Penicillin, Raw milk, Double beam UV/visible Spectrophotometer.

INTRODUCTION

From newborns to the elderly, bovine milk is one of the most popular foods ingested by all populations. Consequently, chemical contaminants, such as veterinary drug residues, must not be present in milk or milk products intended for human consumption (Tremonte *et al.,* 2014).

Antibiotics are used to treat and prevent several diseases caused by infectious agents in both humans and animals. Since the discovery of the first antibiotic in the 1940s, the veterinary fields and healthcare

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industries have depended on them (Groot and Van't Hooft, 2016).

Antibiotic residues could reach milk directly or indirectly in the veterinary field, which uses antibiotics as growth promoters or therapeutic agents for the treatment of bacterial infections and in prophylaxis (Nisha, 2008). They are used in dairy cattle production primarily to treat or prevent disease, and to a lesser extent to increase milk production or improve feed efficiency. Antibiotics used as growth promoters are administered at low doses for extended periods, while as prophylactics, antibiotics are used at low doses to prevent disease (Gustafson, 1991).

Antibiotic residues in milk are unacceptable because of the potential human health hazard and their interference with the manufacturing process, inhibiting yogurt and cheese starter cultures (Beyene, 2016). Antibiotic residues in milk are closely linked to several factors, including the animal's illness state, the type and quantity of antibiotics administered, the type of vehicle used in antibiotic formulations, and the rate of milk production at the time of treatment (Mercer *et al.,* 1970). The most frequent cause of antibiotic residues in milk is intramammary infusion for the treatment of mastitis (92%), followed by injections (6%), and other sources (2%) (Chowdhury *et al.,* 2015).

The presence of these residues is usually attributed to non-observance of withdrawal periods before the sales of edible animal food products, and also due to undesirable practices, such as unregulated and indiscriminate use of drugs and lack of awareness on the rational usage of antibiotics (Kabir *et al.,* 2004). Their appearance in milk produces possible health effects, including hypersensitivity reactions, along with development of antibiotic resistance and cancer (Hassan *et al*., 2014), mutagenicity, nephropathy (Gentamicin), hepatotoxicity, bone marrow toxicity (Chloramphenicol), autoimmunity, immune-pathological effects, carcinogenicity (Sulphamethazine, Oxytetracycline, Furazo-lidone), and allergy (Penicillin) (Nisha, 2008).

In food animals, several antibiotics are extensively used, including beta-lactams and tetracycline. Widespread use of antibiotics would cause residues to appear in milk obtained from antibiotic-treated animals as non-altered parent form or as metabolite and/or conjugate (Ibrahim *et al*., 2009).

All regulatory agencies, consumers, dairy farmers, and milk processors are critically concerned about the health risks to humans posed by the presence of antibiotic residues in milk. To ensure food safety for consumers, several regulatory authorities around the world, including the European Food Safety Agency, Food and Drug Administration, and USA Codex Alimentarius, determined safe levels of antibiotic residues in milk for the protection of the consumer (Adewuyi *et al.,* 2011). But still the presence of antibiotic residues in milk above the Maximum Residual Limit has been recognized by various public authorities and researchers (Sachi *et al.,* 2019).

For the significance of the issue, this research aimed to milk sample screening qualitatively and quantitatively for antibiotic residues, specifically oxytetracycline and penicillin. Also, their risk assessment in adults and children from raw milk was evaluated by calculating Estimated Daily Intake and Hazard Quotient.

MATERIALS AND METHODS

1- Collection of samples:

One hundred and twenty random milk samples were collected during the period from August 2023 to April 2024 in Assiut City, Egypt, from dairy shops (30 samples for each summer and winter), dairy farms and farmers' houses milk (30 samples of each). These samples were collected in clean dry glass bottles and transported to the laboratory to be examined. Dairy shops milk samples were subjected to the Storch test to detect heat-treated samples, according to Lampert (1975).

2- Detection and determination of the residues by double beam UV/visible spectrophotometer.

Sample analysis:

All the collected milk samples (120 raw milk samples) were examined for residues of oxytetracycline and penicillin. Samples were analyzed at the Food Safety Lab, Faculty of Veterinary Medicine, Assiut University using a Double beam UV/visible spectrophotometer, (6850 Jenway, United Kingdom). The used method was according to CODEX Pharmaceutical analysis modern methods (Codex Alimentarius, 1984).

Milk Sample Clean-up:

Five ml of each milk sample and 2.5 ml of 0.1 M succinic acid (pH 4) were vortexed for 10 seconds. Ten milliliters (10 ml) of Mcllvaine-EDTA (0.1 M sodium EDTA, 0.1 M Citric Acid, 0.2 M di sodium hydrogen phosphate Na_2HPO_4) buffer at pH 4 was also added to the vortexed mixture and was sonicated for 10 minutes and then placed in a freezer for 15 minutes. This mixture was then centrifuged at 4000 rpm at 10°C, producing a clear supernatant. The supernatant was filtered with Whatman filter paper (110 mm) and stored at 4°C until analysis (Samanidou and Nisyriou, 2008).

Preparation of standard solutions:

Oxytetracycline standard preparation method:

0.1 mol HCl was prepared and used as the diluent. Five different concentrations (0.0, 0.02, 0.04, 0.06 and 0.08) mg/ml of the oxytetracycline standard were prepared.

Table A: Concentration of standard prepared for oxytetracycline.

S/N	Concentration of standard (mg/ml)	Absorbance	Wavelength (nm)
1	0.00	0	327
2	0.02	0.854	327
3	0.04	1.522	327
4	0.06	2.23	327
5	0.08	2.985	327

Preparation of working concentration of oxytetracycline standard

Each of the concentrations of the standards was subjected to analysis in the Spectrophotometer UV-VIS double beam with the diluent in the blank control beam. The absorbencies of all five different concentrations were recorded after peaking at 327 nm as shown in Table A. A linear graph of concentration and absorbance was then plotted using Excel and the r value was determined r=0.9985.

From the linear curve in the figure, the exponential concentration is 0.04 mg/ml, and this was used as the standard working concentration.

Evaluation of the samples:

Approximately 0.5 ml of standard working concentration (0.04 mg/ml) was pipetted and dispensed into a 10 ml test tube. Two ml of each prepared raw milk sample was added, and the solution was topped to 5 ml with the diluent (0.1 mol HCl). This was then transferred into the cuvette and inserted into the machine for analysis. The absorbance of each sample was recorded after peaking at a wavelength of 327 nm and the concentration of the residue was calculated using the equation from the linear curve according to Beer Lambert's law as thus:

$$y = 36.73x + 0.049$$

Where, y = absorbance.

x = concentration.

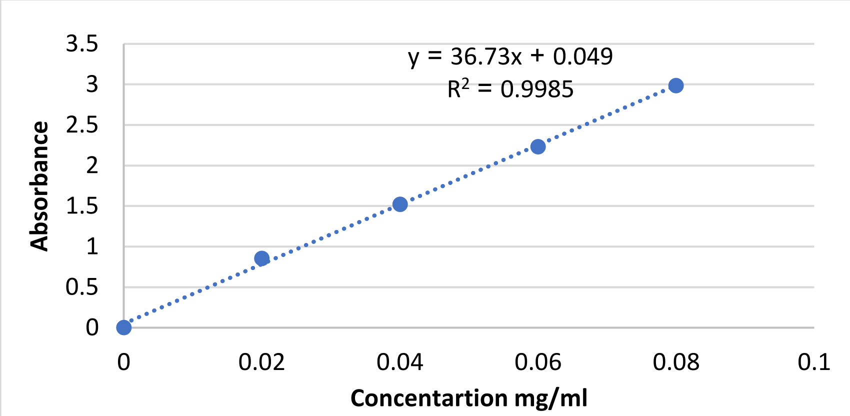

Figure A: Oxytetracycline analytical standard calibration curve (Linear graph)

Penicillin standard preparation method: Approximately 0.1 mol of HCl was prepared and used as the diluent. Five different concentrations of the penicillin standard (0.00, 0.03, 0.06, 0.125 and 0.25) mg/ml were prepared, Table B.

Table B: Concentration of standards prepared for penicillin.

S/N	Concentration of standard (mg/ml)	Absorbance	Wavelength (nm)
1	0.00	0	327
2	0.03	0.109	327
3	0.06	0.208	327
4	0.125	0.497	327
5	0.25	0.987	327

Preparation of working concentration of penicillin standard

Each of the concentrations of the standards was subjected to analysis in the UV-VIS double beam with the diluent in the blank control beam. The absorbance of all five different concentrations was recorded after peaking at 327 nm as shown in Table B. A linear graph of concentration and absorbance was then plotted using Excel and the r value was determined. r = 0.999. From the linear curve, the exponential concentration is 0.125 mg/ml, and this was used as the standard working concentration, Figure B.

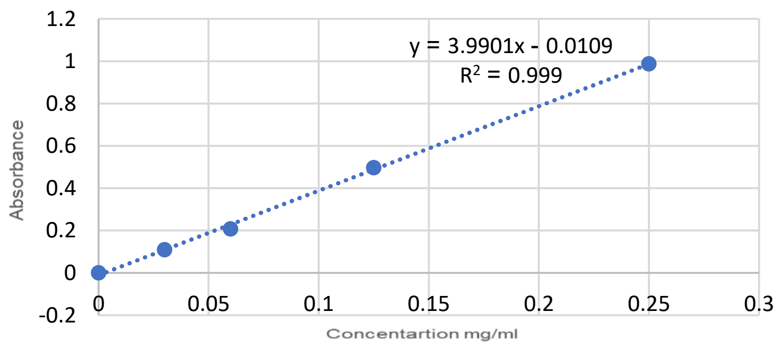

Figure B: Penicillin analytical standard calibration curve (Linear graph).

From the linear curve in the figure, the exponential concentration is 0.125 mg/ml, and this was used as the standard working concentration.

Evaluation of the samples:

Approximately 0.5 ml of standard working concentration (0.125 mg/ml) was pipetted and dispensed into a 10 ml test tube.

Afterward, 2 ml of each prepared raw milk sample was added, and the solution was topped to 5 ml with the diluent (0.1 mol HCl). This was then transferred into the cuvette and inserted into the machine for analysis. The absorbance of each sample was recorded after peaking at a wavelength of 327 nm and the concentration of the residue was calculated using the equation from the linear curve according to Beer Lambert's law as thus:

$$y = 3.9901x - 0.0109$$

Where y = absorbance.
x = concentration.

Human health risk assessment was performed by calculating estimated daily intakes (EDIs). The EDI of residues was calculated for both antibiotics using the following equation (Juan *et al.,* 2010).

$$\textbf{EDI} = \frac{C \times F}{W}$$

Where C= means antibiotic residue concentration in milk (µg/kg), F availability of milk per person, W= mean human body weight (Adult 60 Kg and child 15 kg).

The Hazard Quotient was calculated using the following equation:
Hazard Quotient = Estimated daily intake/accepted daily intake.

A hazard quotient value of more than one indicates a significant risk to human health, while less than or equal 1 indicates no significant health risk (Rahman *et al.,* 2021).

RESULTS

Table 1: Concentration of oxytetracycline residues (µg/L) in the examined raw milk samples.

Samples	No of the examined samples	Oxytetracycline residues (µg/L)				
		Positive samples.		Concentration. (µg/L)		
		No.	(%)	Min	Max	Average
Summer dairy shops milk	30	21	70	7	17.18	9.78
Winter dairy shops milk	30	25	83.3	3.5	17.67	5.71
Farm milk	30	29	96.6	6	38.65	13.45
Farmers' houses' milk	30	28	93.3	3	11.83	6.69
Total	120	103	85.8	3	38.65	8.99

Table 2: Concentration of penicillin residues (µg/L) in the examined raw milk samples.

Samples	No of the examined samples.	Penicillin residues (µg/L).				
		Positive samples.		Concentration. (µg/L)		
		No.	(%)	Min	Max	Average
Summer dairy shops milk	30	28	93.3	17	94.7	43
Winter dairy shops milk	30	26	86.7	5.9	212	66
Farm milk	30	25	83.3	51.8	250	145
Farmers' houses' milk	30	27	90	35	117	57.5
Total	120	106	88.3	5.9	250	76.5

Table 3: Oxytetracycline residue in the examined samples in relation to the maximum residual limit (MRL)*.

Samples	Oxytetracycline residue (µg/L)			
	Positive samples within MRL*		Positive samples above MRL*	
	No.	(%)	No.	(%)
Summer dairy shops milk	21	100	0	0
Winter dairy shops milk	25	100	0	0
Farm milk	29	100	0	0
Farmers houses' milk	28	100	0	0
Total	103	100	0	0

*MRL of oxytetracycline in milk: 100 µg/L, recommended by **(Codex Alimentarius, 2003).**

Table 4: Penicillin residue in the examined samples in relation to the maximum residual limit (MRL)*.

Samples	Penicillin residues (µg/L)			
	Positive samples within MRL*		Positive samples above MRL*	
	No.	(%)	No.	(%)
Summer dairy shops milk	0	0	28	100
Winter dairy shops milk	0	0	26	100
Farm milk	0	0	25	100
Farmers houses' milk	0	0	27	100
Total	0	0	106	100

*MRL of penicillin in milk: 4 µg/L, recommended by **(Codex Alimentarius, 2012).**

Table 5: Raw milk samples contaminated with residues of one or two of the tested antibiotics.

Samples	Free samples		One Antibiotic		Two Antibiotics	
	No.	(%)	No.	(%)	No.	(%)
Summer dairy shops milk	2	6.67	6	20	22	73. 33
Winter dairy shops milk	2	6.67	5	16.67	23	76.67
Farm milk	1	3.33	4	13.33	25	83.33
Farmers houses' milk	1	3.33	3	10	26	86.67
Total	6	5	18	15	96	80

Table 6: Risk assessment of oxytetracycline residue from raw milk in adults.

Samples	Average values	*EDI in adults	Hazard Quotient
Summer dairy shops milk	9.78	32.6	1.086
Winter dairy shops milk	5.71	19	0.633
Farm milk	13.45	44.8	1.49
Farmers houses' milk	6.69	22.3	0.743
Total	8.99	29.96	0.99

ADI for oxytetracycline: 30 µg/kg **(APVMA, 2016).**
*EDI: Estimated daily intake from consumption of 200 ml milk per day.

Table 7: Risk assessment of oxytetracycline residue from raw milk in children.

Samples	Average values	*EDI In children	Hazard Quotient
Summer dairy shops milk	9.78	130.4	4.35
Winter dairy shops milk	5.71	76.13	2.54
Farm milk	13.45	179.3	5.98
Farmers houses' milk	6.69	89.2	2.97
Total	8.99	119.86	3.99

ADI for oxytetracycline: 30 µg/kg **(APVMA, 2016).**
*EDI: Estimated daily intake from consumption of 200 ml milk per day.

Table 8: Risk assessment of penicillin residue from raw milk in adults.

Samples	Average values	*EDI in adults	Hazard Quotient
Summer dairy shops milk	43	143.3	0.72
Winter dairy shops milk	66	220	1.1
Farm milk	145	483.3	2.4
Farmers houses' milk	57.5	191.67	0.96
Total	76.5	255	1.275

ADI for penicillin = 200 µg/kg **(APVMA, 1995).**
*EDI: Estimated daily intake from consumption of 200 ml milk per day.

Table 9: Risk assessment of penicillin residue from raw milk in children.

Samples	Average values	*EDI in children	Hazard Quotient
Summer dairy shops milk	43	573.3	2.87
Winter dairy shops milk	66	880	4.4
Farm milk	145	1933.3	9.67
Farmers houses' milk	57.5	766.67	3.83
Total	76.5	1020	5.1

ADI for penicillin = 200 µg/kg **(APVMA, 1995)**.
*EDI: Estimated daily intake from consumption of 200 ml milk per day.

DISCUSSION

Milk is a primary source of nutrients in diets around the world and is one of the most essential foods for human nutrition. It is considered a complete food that contains all the macronutrients, in addition to trace elements (Buldini *et al*., 2002). On the other hand, milk may be a potential ready source for chemical contaminants, such as antibacterial drugs (Khaniki, 2007).

Antibiotics are used in therapeutics and prophylaxis of infectious diseases, or as a production aid in food animals (Chauhan *et al*., 2018). The most used antimicrobial classes in food animals include beta-lactams and tetracyclines (Mitchell *et al.,* 1998; Priyanka *et al.,* 2017).

Results recorded in Table 1 revealed that the incidence of oxytetracycline residue in positive samples was 85.8%, with an average concentration of 8.99 µg/L. The highest incidence was in farm milk (96.6 %), followed by farmers houses' milk (93.3%), winter dairy shops milk (83.3%), and summer dairy shops milk (70 %). The highest average concentration was also in farm milk samples (13.45 µg/L). Notably, the average concentration in summer dairy shops milk (9.78 µg/L) was higher than in winter dairy shops milk (5.71 µg/L). The highest incidence and concentration in farm milk samples could be attributed to the overuse of antibiotics to treat and prevent diseases in dairy farms (Abebew *et al*., 2014). The appearance of antibiotic residues in animal products was due to the excessive and irrational administration of antibiotics to farm animals (Gajda *et al.* 2012 and Ronquillo and Hernandez, 2017).

The obtained results of the total incidence of oxytetracycline residues were in harmony with Beltrán *et al.* (2013), Abebew *et al.* (2014), but higher than Hebbal *et al.* (2020), Al-Shaalan *et al.* (2022), Raza *et al.* (2022) and Abdel Wahab *et al.* (2024). Also, higher than the incidence of tetracycline residues obtained by Sachi *et al.* (2019), Brown *et al.* (2020) and Zeghilet *et al*. (2022).

The obtained results of the average concentration of total positive samples were lower than Abo El-Makarem *et al.* (2020), Al-Shaalan *et al.* (2022), Raza *et al.* (2022), but were in harmony with Dimitrieska-Stojkovic *et al*. (2011) while higher than Buczkowska *et al.* (2021).

Misuse of antibiotics can lead to pollution that enters the environment in an active form, which is another worry. Because humans and animals excrete more than 70% of tetracycline antibiotics (Daghrir and Drogui, 2013).

When treated for mastitis, the elimination of tetracycline was slower in elderly cows.

In the case of mastitis treatment, older cows were slower to eliminate tetracycline (Siljanoski *et al*. 2018). Therefore, consuming milk from an animal treated with tetracycline and developing subclinical mastitis should be restricted to at least 4 days after milk discharge (Magon *et al.* 2018). As the withdrawal time of oxytetracycline in milk is 96 hr after the last treatment (Baynes *et al*. 2016).

Documented results in Table 2 reported that the incidence of penicillin residue was 88.3 % with an average concentration of 76.5 µg/L. The highest incidence was in summer dairy shops milk (93.3%) followed by farmers' houses milk (90%), winter dairy shops milk (86.7%) and farm milk (83.3 %). The highest average concentration was also in farm milk samples (145 µg/L). Notably, the average concentration in winter dairy shops and farmers' houses milk (66 and 57.5 µg/L) were higher than summer dairy shops milk (43 µg/L). Owners treating sick cows without seeking professional advice, inadequate documentation and ignorance of drug withdrawal times, improper care of dairy cows, and low awareness are the reasons for the presence of antibiotic residues in milk (Abebew *et al.,* 2014). Hamdan, (2019) reported that autumn had the highest incidence (67 %) and the lowest in summer (13 %) in raw milk samples. It was determined that the probability of detecting antibiotics in milk during spring and autumn is higher than other seasons (Kaya and Filazi, 2010). Moreover, it was noted that in autumn and winter, mastitis occurs more frequently due to climatic changes, and as a result, antibiotic therapy is carried out more often (Grădinaru *et al*., 2011; Rassouli *et al*., 2014).

The obtained results of total incidence of penicillin residues were similar to Kaya and Filazi, (2010) and higher than Olatoye *et al.* (2016), Priyanka *et al.* (2019) and Zeghilet *et al*. (2022). The recorded results of the average concentration of total positive samples were lower than Ghidini *et al.* (2003) and Kumar *et al.* (2022), while higher than Khaskheli *et al*. (2008), Thapaliya *et al*. (2013) and Olatoye *et al*. (2016).

Many allergic reactions, such as serum sickness and anaphylaxis, are linked to antibiotic residues, particularly in the case of penicillin (Riedl and Casillas 2003; Beyene, 2016). In people who are sensitive, penicillin residues in milk may cause allergic responses (Martins-Junior *et al*., 2007).

The high concentration of penicillin residue found in raw milk may be related to the fact that injectable penicillin preparations intra-mammary or systemically are mostly used by farmers and herdsmen to prevent or treat mastitis in lactating cows. This pattern has been followed for a long period (Pyörälä, 2009; Mangesho *et al.,* 2017 and Ogunshe and Adeola, 2019). The high concentration of penicillin residue in raw milk may possibly be related to the fact that farmers and herdsmen usually dose their cows with these chemotherapeutic agents as soon as after parturition for prevention or treatment post-partum complications or mastitis (Priyanka *et al.,* 2017).

To ensure the safety of food intended for human consumption, most countries have established official standard laws aimed at strictly regulating the maximum residue levels (MRLs) of veterinary drugs in animal products (Rana *et al.,* 2019). When starter fermentation is inhibited during the manufacturing of cheese and yogurt, antibiotic residues above the maximum recommended level (MRL) can result in significant losses for the fermented dairy products business. (Molina *et al.* 2003 and Sachi *et al.* 2019).

Comparing the indicated results of oxytetracycline residue with the MPL, Table 3 showed that all positive milk samples were below the maximum permissible limit which is 100 µg/L according to Codex Alimentarius (2003).

Results of oxytetracycline residue samples agree with those obtained by Malgwi *et al.* (2023) and Rahman *et al*. (2024). However, the results were in disagreement with Kaya and Filazi (2010), who found that the oxytetracycline residues were all above the MPL, and higher incidence of positive samples for oxytetracycline exceeding maximum residual limits were obtained by Abebew *et al*. (2014), who reported that 83.33 % of positive milk samples contained oxytetracycline residues above the MPL. Also, Moudgil *et al.* (2019) found that 1.65 % of the milk samples had more oxytetracycline residues than the maximum recommended levels, while Gaurav *et al.* (2014) indicated tetracycline residues (2.3 %) above MPL.

Comparing the presented results of penicillin residue with the MPL in Table 4 showed that all positive milk samples were above the maximum permissible limit, which is 4 µg/L, according to Codex Alimentarius (2012).

The obtained results for penicillin were in agreement with Kaya and Filazi (2010), Olatoye *et al.* (2016) and Malgwi *et al.* (2023). Also, Abebew *et al*. (2014) showed that 16.66 % of penicillin residues above MPL. In contrast with Kabrite *et al*. (2019) and Buczkowska *et al.* (2021), who reported that all positive samples contain penicillin residues below the MPL. Consumers exposed to antibiotic residues exceeding Maximum Residual Limits (MRL) may have different harmful effects, like allergic reactions or disruption of their gut microbiota, among other negative consequences (Stolker *et al.,* 2007).

Illustrated data in Table 5 declared that the percentage of total raw milk samples unable to find antibiotic residues was 5 %, as 6.67, 6.67, 3.33 and 3.33 % in the summer dairy shops, winter dairy shops, farm and farmers' houses milk samples respectively. While the total percentage of samples having just one antibiotic contamination was 15 % as 20, 16.67, 13.33 and 10 % in the summer dairy shops, winter dairy shops, farm and farmers' houses milk samples, respectively. Finally, the percentage of samples contaminated with the two antibiotic residues was 80 % as 73.33, 76.67, 83.33 and 86.67 % in the summer dairy shops, winter dairy shops, farm and farmers' houses milk samples, respectively.

Pogurschi *et al.* (2015) recorded that 92.4 % of examined samples were contaminated with residues of one antibiotic, while 7.58 % were contaminated with more than one antibiotic. Abo El-Makarem *et al.* (2020) reported that 60% of each cow and buffalo milk samples were free from antibiotics, while ١٥ and 20% were contaminated with only one antibiotic, and 20 % each were contaminated with two antibiotic residues, and 5% cow milk samples were contaminated with three antibiotic residues. Finally, Oruç and Sonal (2005) failed to detect oxytetracycline or penicillin residues in all examined raw cow milk samples in Bursa, Turkey.

Antibiotics are widely used for the prevention and treatment of diseases in dairy farms, especially tetracyclines and penicillin. G. oxytetracycline was the first antibiotic used in most farms 46.74%, followed by penicillin (36.96%), according to the respondents (Abebaw *et al.,* 2014). Misuse of antibiotics has resulted in the evolution of superbugs that are resistant to several drugs. This has increased the incidence of morbidity, failed treatments, and increased healthcare costs (Brown *et al*., 2020).

The data summarized in Table 6 showed the risk assessment of oxytetracycline residues from raw milk in adults through the calculation of the Estimated Daily Intake (EDI) and compared to the Acceptable Daily oxytetracycline Intake (ADI) (30 µg/kg) recommended by APVMA (2016). The average of EDI of oxytetracycline in adults of total positive samples was 29.96 µg/kg BW/day which was within the ADI. The highest EDI of oxytetracycline in summer dairy shops milk and farm milk samples were 32.6 and 44.8 µg/kg bw/day were above ADI. On the other side, the lowest EDI of winter dairy shops milk and farmers houses' milk samples were 19 and 22.3 µg/kg BW/day which were below the ADI. The Hazard Quotient of an average of total positive raw milk samples in adults was 0.99, which was less than one, as if the HQ is ≤ 1, the risk to human health exposure is not significant; if HQ is > 1; the consumer is at risk (Rahman *et al*. 2021). In the summer dairy shops milk and farm milk, samples were 1.086 and 1.49, which were above one HQ, but the HQ of winter dairy shops milk samples and farmers houses' milk samples were 0.633 and 0.733, which were below the one HQ as they have no significant health risk from consumption of such milk.

Widiastuti *et al*. (2023) detected that the EDI in adults was lower than ADI (0.0395 and 0.0165 µg/kg BW/day) and the HQ as 0.0033 and 0.0006 in oxytetracycline and tetracycline respectively. Al-Shaalan *et al.* (2022) demonstrated EDI of oxytetracycline in adults as 0.194, 0.213, 0.228 and 0.242 µg/kg and the HQ was very low as 0.0065, 0.0071, 0.0076 and 0.0081.
Results in Table 7 discussed the risk assessment for oxytetracycline residue from raw milk in children. The average EDI of oxytetracycline in children was 119.86 µg/kg BW/day, which was above the Acceptable Daily Intake. The EDI of oxytetracycline were 130.4, 76.13, 179.3 and 89.2 µg/kg BW/day in the summer and winter dairy shops, farm and farmers' houses milk samples which were above the ADI.

The Hazard Quotient of total raw milk samples in children was 3.99, which was more than one. The HQ were 4.35, 2.54, 5.98 and 2.97 in the summer dairy shops, winter dairy shops, farm and farmers' houses milk samples which were above the normal HQ, and it may cause risk to the children consuming such milk. Contrary to Aalipour *et al.* (2015), Moudgil *et al.* (2019), Al-Shaalan *et al.* (2022) and Widiastuti *et al.* (2023), who detected that the EDI of oxytetracycline in children was below ADI and the HQ was less than 1.

Because tetracyclines include a risk of secondary tooth discoloration, they should not be used by pregnant women or children under the age of eight. Nephrotoxicity, hepatotoxicity, darkening of the skin in sun-exposed areas, and hypersensitivity reactions are additional long-term consequences. Additionally, hypokalaemia and proximal and distal renal tubular acidosis have been linked to tetracyclines (Goldfrank *et al.* 2002).

In Table (8) the results showed the risk assessment for penicillin residues from raw milk in adults by comparison between the Estimated Daily Intake (EDI) of penicillin and its Acceptable Daily penicillin Intake (ADI) (200 µg/kg) recommended by APVMA (1995). The average EDI of penicillin in adults of total positive samples was (255 µg/kg BW/day) which was above the Acceptable Daily Intake. The EDI of penicillin below ADI were 143.3 and 191.67 µg/kg BW/day in summer dairy shops and farmers' houses milk samples, while 220 and 483.3 µg/kg BW/day in winter dairy shops, farm milk samples were above the ADI.

The Hazard Quotient of an average total positive raw milk sample in adults was 1.275, which was more than one, the HQ was below one as 0.72 and 0.96 in the summer dairy shops and farmers houses' milk samples but were 1.1 and 2.4 in the winter dairy shops and farm milk samples, which is above one. Our findings of the EDI results and HQ were in opposition to that obtained by Kabrite *et al.* (2019); Abo El-Makarem *et al.* (2020) and Pogurschi *et al.* (2022), who detected that EDI of penicillin was below ADI.

The illustrated results in Table 9 recorded the risk assessment for penicillin residues from raw milk in children. The average EDI of penicillin in children of a total positive sample was (1020 µg/kg BW/day) which was above the Acceptable Daily Intake. The EDI of penicillin were 573.3, 880, 1933.3 and 766.67 µg/kg BW/day in summer and winter dairy shops s, farm and farmers houses' milk samples which were above the ADI.

The Hazard Quotient of an average total positive raw milk sample in children was 5.1, which was more than one. The HQ were 2.87, 4.4, 9.67 and 3.83 in the summer dairy shops, winter dairy shops, farm and farmers' houses milk samples, which were above one, which clearly shows the high risk for children from drinking such milk. Our results of ADI and HQ were higher than Kumar *et al.* (2022).

CONCLUSION

From the aforementioned results, it is noticed that all positive samples for oxytetracycline were within MPL, while all positive samples for penicillin were above MPL. Risk assessment for the tested antibiotics showed health risks for adults and higher risks for children from consuming such contaminated milk. So restrictions should be imposed on the accessibility of veterinary drugs to farmers and unspecialized workers to avoid the uncontrolled use of antibiotics.

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تقييم مخاطر بقايا الأوكسي تيتراسيكلين والبنسلين في اللبن الخام

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تشكل بقايا المضادات الحيوية في اللبن مصدر قلق كبير للصحة العامة حيث يتم استهلاك اللبن على نطاق واسع من قبل الرضع والشباب والبالغين في جميع أنحاء العالم. هدف هذه الدراسة هو تحديد وجود وتركيزات بقايا أوكسي تتراسيكلين والبنسيلين في عينات اللبن الخام حيث إنها الأكثر استخداما في المجال البيطري. تم جمع مائة وعشرون عينة عشوائية من اللبن الخام (٦٠ عينة خلال فصلي الصيف والشتاء من محلات الألبان المختلفة و٣٠عينة من مزارع الألبان و٣٠ عينة من منازل الفلاحين) من مناطق مختلفة في مدينة أسيوط، مصر. حيث تم جمع العينات في أوعية زجاجية نظيفة ومعقمة ثم نُقلت الى المعمل للكشف عن بعض بقايا المضادات الحيوية (أوكسي تتراسيكلين والبنسيلين) تم هضم كل العينات وتحليلها في معمل سلامة الغذاء كلية الطب البيطري بجامعة أسيوط باستخدام جهاز مقياس الطيف الضوئي للأشعة فوق البنفسجية / المرئية مزدوج الحزمة لتحديد وقياس بقايا الأوكسي تيتراسيكلين والبنسيلين .

ويمكن تلخيص النتائج المتحصل عليها على النحو التالي: كانت نسبة العينات الإيجابية لأوكسي تتراسيكلين ٢١ و٢٥ و٢٩ و٢٨ ٪ على التوالي في العينات المجمعة من محلات الألبان خلال فصل الصيف والشتاء ولبن المزارع ولبن منازل الفلاحين. بينما كانت نسبة العينات الإيجابية للبنيسيلن ٢٨ و٢٦ و٢٥ و٢٧ ٪. بالنسبة للحد الأدنى والأقصى لبقايا الأوكسي تتراسيكلين لأجمالي عدد العينات كان ٣ و٣٨,٦٥ ميكروجرام/لتر وبلغت قيمة المتوسط ٨,٩ ميكروجرام/لتر. بالنسبة للحد الأدنى والأقصى لبقايا البنيسيلن لأجمالي عدد العينات كان ٥,٩ و٢٥٠ ميكروجرام/لتر وبلغت قيمه المتوسط ٧٦,٥ ميكروجرام/لتر. كانت جميع نسب العينات الإيجابية التي تحتوي بقايا أوكسي تتراسيكلين أقل من الحدود المسموح بها (MRL)١٠٠ ميكروجرام/لتر. بينما كانت جميع نسب العينات الإيجابية التي تحتوي على بقايا البنسيلين أعلي من الحدود المسموح بها (MRL) ٤ ميكروجرام/لتر.

من إجمالي عدد العينات ١٢٠ عينة، كانت ٦ عينات خالية من أي بقايا مضادات حيوية، و١٨ عينة بها بقايا مضاد حيوي واحد، و٩٦ عينة بها بقايا المضادين الحيويين. وأيضا تم تقييم مخاطر بقايا الأوكسي تيتراسيكلين والبنسيلين في البالغين والأطفال في عينات اللبن الخام من خلال حساب قيم مقدار التناول اليومي ومعامل الخطر.

هذا وقد تم ذكر الاشتراطات الصحية الواجب اتباعها لدرء خطر هذه البقايا على صحة المستهلك.