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EVALUATION OF SOME HARMFUL RESIDUES IN MILK

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ABSTRACT

A total of 90 randomly collected milk samples from different localities in Ismailia Governorate for determination of some (antibiotic residues, heavy metals and chemical preservatives). Results revealed that qualitative detection of β-lactams and fluoroquinolones residues in milk samples by using Airflow™ Strip Test Kit were 42.2% and 21.1%, respectively. Quantitative detection by HPLC analysis revealed that 32.2%, 7.8 % and 21.1 % of examined milk samples were contaminated with Amoxicillin, Ceftrixone and ciprofloxacin, respectively with the mean concentrations of 6.95± 1.36 ,8.67±2.42 and 8.68 ±1.9 ppb, respectively. The antibiotic residues after boiling was significant decreased to 1.04 ppb, 1.09 ppb and 6.33 ppb for amoxicillin, ceftriaxone and ciprofloxacin residues, respectively with the reduction percent of 85%, 87.4% and 27%, respectively. Heavy metals were detected by using atomic absorption spectrometer. The incidence of lead in examined milk samples were 75.5% with the mean concentration 0.4555± 0.0828ppm. 7.77 % of all examined milk samples shown to be adulterated with formalin. These contaminants constitute a public health hazards to many consumers and attention should be pay to prevent reaching of such contaminant to consumers.

INTRODUCTION

Milk is a primary source of nutrients in diets all around the world and is one of the most essential food for human nutrition, It considered as 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 disease agents and other biological and chemical contaminants. Animal products and byproducts can potentially be contaminated with thousands of chemicals such as antibacterial drugs, industrial chemicals and heavy metals **(Khaniki, 2007).**

The presence of antibiotic residues in food products especially milk implicates certain damages in the public health and leads to economic losses to dairy industry **(Hou *et al*., 2014),** the growth of resistant bacterial strains and imbalances in intestinal microflora **(Borràs *et al*., 2011)**. Low concentrations of antimicrobial drug residues create problems in the manufacturing of milk products by inhibiting the starter cultures **(Stead *et al*., 2008).** So many countries have imposed regulatory limits (maximum residue limit/MRL) that allowed by the regulatory authorities to prevent sanitary hazard for the consumers and without effect on the manufacturing processes **(Paturkar *et al.,* 2005).**

The identification and quantification of antibiotic residues in milk was carried out using high performance liquid chromatography (HPLC) which produce accurate information about the prevalent status of antibiotic residues. It is generally recommended that one or more a screening assays, in addition to HPLC methods, should be used as a protocol for determination antibiotic residues in foods **(Gaurav, 2014).**

Milk may be contaminated by heavy metals that at certain levels may cause toxicity to consumers. This toxicity is attributed to accumulation of heavy metals in the body which are not metabolized to extractable products**;** Heavy metal residues in milk are of particular concern because milk are largely consumed by infants and children. The toxic metal content of milk and dairy products depend on several factors, in particular environmental conditions, the manufacturing process and the possible contamination during several steps of the manufacturing processes (**Temiz and Soylu,2012).** There are various analytical techniques to measure the amount of metals contamination in milk and dairy products. One of the best and most efficient methods is atomic absorption **(Mahmoudi *et al.,* 2017).**

Milk fraud is one of the most serious food safety issues that, the dairy sector is facing today, which causes a potential adverse human health effects associated with consumption of adulterated milk contained chemical preservatives such as formalin, hydrogen peroxide and boric acid which are used to preserve food by preventing growth of microorganism to increase the storage period of milk, and to enhance the shelf life of milk .The adulteration of milk deteriorates quality of milk may cause serious problems for human health. Like; gastrointestinal problems including gastric ulcer, diarrhea, colon ulcer and kidney problems etc. **(Zubeir *et al.,* 2009 and Barham *et al.,* 2014).**

The present work was planned out as an attempt to throw some spotlights on evaluation of some harmful residues in milk and methods of minimize antibiotic residues in milk.

MATERIAL AND METHODS

Sampling:

A total of 90 random milk samples were collected from different localities in the Ismailia governorate during the period from November 2016 to December 2017. Samples of raw and fresh milk purchased from local retailed commercial markets, shops and street vendors. Each samples weighted 1500 ml and divided in three portions (500 ml), each in plastic bag or capped plastic bottle then identified and transferred in icebox to the laboratory with a minimum delay under refrigeration conditions for residues determination. First portion was sent to laboratory of Animal Heath Research Institute (AHRI "Dokki, Giza") for detection of antibiotic residues. Second portion was sent to laboratory of department of chemistry, Faculty of science - Suez Canal University for detection of heavy metals and the third portion was sent to laboratory of department of Food Hygiene, Faculty of Veterinary Medicine -Suez Canal University for detection chemical preservatives.

Determination of antibiotic residues:

The detection of antibiotic residues in milk was carried out using the protocol recommended by **E.U. (2002) /657/ EC legislation**, which requires two stages:

Qualitative detection of antibiotics residues:

In this experiment colloidal gold-based immune chromatographic assay for the detection of the antibiotic residues were done by using two type of commercial kits that have been obtained from Bioo Scientific Corporation Company**. Roko *et al.,* (2015)** have been used the same commercial kits. Detection limits were done by visual interpretation method.

a) AuroFlow™ β-lactam Strip Test Kit for Milk has the capacity for 96 Catalog #: 1067-01 & 1067-01.

b) The AuroFlow™ Fluoroquinolone Strip Test Kit for Milk has the capacity for 96 (Cat # 1102-01.

Quantitative detection of antibiotics residues:

Milk samples tested positive by the colloidal gold-based immune chromatographic assay (TheAuroFlow™ Strip Test Kit) were analyzed by HPLC for detection of antibiotics residues before and after heat treatment. For the extraction, clean-up and estimation of β-lactam residues the method proposed by **Khaskheli *et al.,* (2008)**.While the method of extraction, clean-up and estimation of fluoroquinolones (Enrofloxacin and Ciprofloxacin) residues, proposed by **(Cinquina *et al.,*(2003) and Dimitrova *et al.,*(2007)** were used with slight modifications. The HPLC system was a constant liquid chromatography pump, Agilent 1200 series co., Germany, with variable wave length detector co., Germany in addition to a software chemistation,Germany.

The mobile phase in β- lactams quantitation was 0.1% trifluroacetic acid and acetonitrile (50:50). It was pumped at a flow rate of 1ml/ min. the Beta lactam was detected at wave length 254 nm, While a mobile phase of fluoroquinolones was Acetonitrile: Water: Phosphoric Acid: Triethlyamine: Tetra butyric acid (20:79:0.3: 0.4:0.3) was used. The flow rate was kept at 0.75 ml/min. Chromatography was performed at 27˚C with Photo Diode Array (PDA) detector set at 270 nm. The total run time was kept at 5 min.

Effect of boiling on antibiotic residues:

The effect of boiling was evaluated according to **Gauarv *et al.,* (2014).** An aliquot of the milk samples (100 ml) was kept in a beaker and subjected to boiling point for 5 minutes over a gas burner. The effect of boiling on the levels of antibiotic residues was evaluated as the percentage reduction of these residues before and after heat treatment of the milk sample contained antibiotic residues.

Determination of heavy metals residues:

Methods of sampling and analysis of heavy metals are laid down in the Commission Regulation **(EC 2007 No.333)**. Digestion procedure and preparation of analyte solution was carried out using " Wet Digestion" method according to **Richards (1968)** and **Ismail *et al.,* (2015).** All filtrated samples were analyzed for their contents according to **Medina *et al.*, (1986)** by using "Perkin Elmer Atomic Absorption Spectrophotometer (AAS) model 2380 equipped with Mercury Hydride System (MHS), USA 1988". Quantitative determination of heavy metals were recorded directly from the digital scale of AAS and they were calculated according to the following equation: Element, (ppm, mg/kg) = R × D/W Where:_R= Reading of element concentration, ppm from the digital scale of AAS. (mg/kg of metal in prepared samples obtained by calibration).

D= Dilution of the prepared sample (final volume of prepared sample in ml). W= Weight of the sample in grams. The registered values for lead, copper and zinc were expressed as µg/g wet weight (ppm).

Detection of Chemical preservatives:

Detection of preservatives in milk was carried out using the specific tests.

Formalin/formaldehyde can be detected in milk By Hehner test reported by **Vishweshwar and Krishnaiah (2005).** Boric Acid and Borates (Boric acid or Borax) were detected in milk by Glycerol test reported by BIS **(IS: 1961)**. Hydrogen Peroxide was detected in milk by Pien's test reported **(Pien's *et al.*, 1953)**. Salicylic acid detection was detected in milk by ferric chloride test reported by **Dixit (2012).**

Statistical analysis: using Minitab 16 software.

RESULTS

Table (1): Incidence of antibiotic residues in examined milk samples by using AuroFlow™ kit (N=90).

Antibiotic group	Positive						Negative	
	Total		Slight		Strong			
	No	%	No	%	No	%	No	%
β-lactam	38	42.2	26	28.9	12	13.3	52	57.8
fluoroquinolones	19	21.1	12	13.3	7	7.8	71	78.9

Table (2): Incidence of some antibiotic residues in examined milk samples determined by HPLC (N=90).

Antibiotic group	Antibiotics	Positive samples		Negative samples	
		No	%	No	%
β-lactams	Amoxicillin	29	32.2	61	67.8
	Ceftriaxone	7	7.8	83	92.2
fluoroquinolones	Ciprofloxacin	19	21.1	71	78.9

Table (3): Concentration of some antibiotic residues (PPb) in examined milk samples determined by HPLC (N=57).

Antibiotic group	Concentration of some antibiotic residues(PPb)			
	Antibiotics	Minimum	Maximum	Mean ±S.E.
β-lactams	Amoxicillin	N.D	7.68	6.95±1.36
	Ceftriaxone	5.1	9.88	8.67±2.42
Fluoroquinolones	Ciprofloxacin	0.87	10.33	8.68±1.90

Table (4): Effect of heat treatment on the levels of Beta-lactam and Quinolones antibiotics residues in milk samples. (N=55).

Antibiotic group	Antibiotics	Before	After	Reduction %
β-lactam	Amoxicillin	6.95	1.04	85%
	Ceftriaxone	8.67	1.09	87.4%
Fluoroquinolones	Ciprofloxacin	8.68	6.33	27%

Table (5): Incidence and concentrations of some heavy metals residues (ppm) in examined raw milk samples (N=90).

Metals	Positive samples		Min.	Max.	Mean±S.E.
	No.	%			
Lead (Pb)	**68**	**75.5**	**ND**	**4.404**	**0.4555±0.0828**
Copper (Cu)	**90**	**100.0**	**0.0139**	**2.836**	**0.4179±0.0508**
Zinc (Zn)	**90**	**100.0**	**0.595**	**10.155**	**2.971±0.241**

Table (6): Incidence of chemical preservatives in the examined milk samples (n= 90).

Preservatives	Positive samples	
	No.	%
Formalin	**7**	**7.77**
Salicylic acid	**0**	**0**
Boric acid	**0**	**0**
Hydrogen peroxide	**0**	**0**

DISCUSSION

Chemical contaminants in milk comprise chemical hazards that may introduce during milk production, dairy processing or packaging.

Veterinary drugs and heavy metals are chemical contaminants that can enter to animal feed and they have some residues in milk. The most contentious residues that occur in milk are antimicrobial drugs. They have some hazards for humans who consume milk and dairy products **Khaniki, (2007)**.

Incidence of antibiotic residues in milk:

Irrational use of drugs in veterinary medicine as well as the need for control of their use becomes even bigger problem when used on food producing animals. In this case, there is the possibility that minimal quantities of drugs and their metabolites (residues) which remain in animal products (milk) induce certain harmful effects in humans as potential consumers of such food **Sanders, (2007)** .

Based on results of (Table 1) some antibiotic residues could be detected in examined milk samples by using colloidal gold basesd immuno chromtography assay (CGICA) commercially called Auroflow strip test. Incidence of β-lactam and fluoroquinolone antibiotic that could be detected in 42.2% and 21.1% of examined samples, respectively.The results were included 12

(13.3%) strong positive and 26 (28.9%) slight positive for β-lactam while 7 (7.8%) strong positive and 12 (13.3%) slight positive for fluroquinolone antibiotics.

The present result is higher than the results that obtained by **Shata *et al.*,(2015) and Movassagh, (2011).**They recorded that the incidence of β-lactams in raw milk samples by using immune chromatographic assay (B star kit) were 12.7% and 4.66% respectively .

The results demonstrated that, the developed method was a suitable for the onsite detection of fluoroquinolones residues in a large number of samples. Also **Sheng *et al.*, (2011)** used immunochromatographic assay using the same batch of the strips to detect fluoroquinolones. Therefore CGIA was an alternative tool for simple, rapid and semi-quantitative detection of fluoroquinolones antibiotics residues in milk on site.

Since this method provides only qualitative and semiquantitative results, the positive samples should be further confirmed by more sensitive methods such as HPLC. In present study a confirmatory technique using HPLC revealed that 32.2%, 7.8 % and 21.1 % of examined milk samples were contaminated with amoxicillin, ceftriaxone and ciprofloxacin, respectively as shown in (Table 2).

There was a considerable difference in positive results obtained by Auroflow strip test and HPLC determination. This difference may be attributed to that AuroFlow™ Strip Test kits was a highly sensitive to wide range of β-Lactams that used in the dairy practice but neither specific nor selective and it cannot identify which antibiotic were present in the milk and may detect antibiotic residues at levels far below the officially mandated safe levels .Although, the most important advantage of qualitative screening tests was that they don't give false negative result but can give false positive responses. Our results were supported by **(Folly *et al.*, 2008)** who reported that the advantage of using a chromatographic method is the possibility of identifying and quantifying the exact value of the antibiotic in the milk. The lack of volatility and thermal instability of many antibiotics makes HPLC the best of choice for analysis and confirmation of veterinary drug residues **(Kennedy *et al.*, 1998**). Antibiotics degrade during storage especially when stored in ambient temperatures **(Marth and Steele, 2001)** and when the milk sample was even slightly spoiled. The β-lactamase enzyme produced by the spoilage bacteria can destroy the β-lactam residues.(**Guay *et al.*, 1987**). Higher results reported by **Layada *et al.*, (2016)** who concluded that 65.46 % of examined milk samples contained antibiotics residues at levels higher than the MRL. **Mahmoudi** ***et al.,*** **(2013)** recorded 57.5% of raw milk samples contaminated with antibiotic residues.

Variable incidence of antibiotic residues could be detected by different authors, 24.7% by **Kouamé-Sina** ***et al.,*** **(2010),** 36% **by Kurwijila** ***et al.,*** **(2006),**respectively. **Ahlberg** ***et al.,*** **(2016)** recorded that (24%) samples were positive (15%) unclear and (61%) negative for antibiotic residues in milk samples.

The incidence of fluoroquinolones antibiotic residues in raw milk samples were nearly similar that reported by **Gaurav, (2014)** who mentioned that a mong the total of pooled milk samples fluoroquinolones were found positive in 17 samples which represent (15.7%) and ciprofloxacin represent (12%). Higher results were reported by **Navratilova** ***et al.,*** **(2011)** who recorded that 87.3% of the raw milk samples were positive for the fluoroquinolones residues. While lower results were reported by **Aguilera-Luiz** ***et al.,*** **(2008) and Nizamlıoğlu and Aydın (2012)** who found that none of the examined milk samples were positive for fluoroquinolones residues. Detection of a high rate of occurrence to amoxicillin could be due to the fact that drugs like amoxicillin are frequently employed in farms leading to selection pressure. The observed high incidence to amoxicillin was not surprising since these antibiotic were commonly used in most of human and food animals for management of bacterial infections. In general, the low rate of occurrence of ceftriaxone was found in the current study. This could be due to the fact that most of the 3rd generation cephalosporins is not a common practice in veterinary medicine. While the occurrence of ciprofloxacin in milk was surprising since these antibiotic not be proven to use in food animals. The occurrence of ciprofloxacin in the present study may be due to the fact that ciprofloxacin is a marker residue for enrofloxacin. Enrofloxacin was approved for use as a veterinary drug and when it is metabolized, its pharmacologically active metabolite; ciprofloxacin; is produced. Hence detection of ciprofloxacin during market screening often reflects the use of both enrofloxacin and ciprofloxacin **(Botsoglou and Fletouris 2001; Navrátilová** ***et al.,*** **2011).**

Estimation of antibiotic residues in milk:

Antibiotic residues in milk samples may be transmitted to the consumers causing many development of antibiotic resistant bacteria in human being which have a public health importance **(Sanders, 2007 and Eguale** ***et al.,*** **2017).** Based on the results in (Table 3) showed the mean concentration of amoxicillin ,ceftriaxone and ciprofloxacin residues in examined milk samples were 6.95+1.36 ,8.67 +2.42 and 8.68 +1.9 ppb, respectively.

Amoxicillin maximum residual limits (MRLs) set by different standards like European Commission **(EC 2010 No. 37**), Egyptian standard **(ES 2010 No.7135).** was 4ppb and 4ppb of each , respectively. 89.1 % of positive milk sample has amoxicillin residues concentration exceeded (MRLs) set by different standards like EC, and ES Egyptain stanards.

Ceftriaxone stipulated by by **ES (2010) , EC (2010)** was 20-120 ppb each. None of examined samples were exceeding the MRL according to EU and Egyptain stanards **(ES 2010 No. 7135).** Ciprofloxacin MRL stipulated by byES ,EC and Japan Food Chemical Research Foundation **JFCRF (Change *et al.,* 2010**) was 100ppb ,100ppb and 50 ppb ,respectively. None of examined samples were exceeding the MRL according to EU ,JFCRF and Egyptain stanards **(2010 No. 7135).**

High antibiotic residues in milk may increase resistant to “critically important antibiotics (Amoxicillin , ceftriaxone and ciprofloxacin) which means developed multi-drug resistant that defined as resistance to at least three classes of antibiotics or resistance to at least one clinically important drug. Which poses a threat to public health and emerging issues.

Our results may support the finding of **Olesen *et al.,* (2004**)**;Wong *et al.,* (2014)** who mentioned that resistance to β-lactam antimicrobials and quinolones have increased dramatically isolates from humans as well as food animals worldwide. Also **Eguale *et al.,* (2017)** observed significantly increased resistance to β-lactam and quinolone classes in isolates from humans and animals. In the current study high presence of the antibiotic residues in contaminated milk samples may support the previous findings that, the degree of contamination of milk and dairy products with antmicrobial residues differs, depending on the level of legislation and effectiveness of methods in different countries **(Kaya and Filazi, 2010**)**.** The risk of residue from the milk was higher in developing countries compared to developed one. This might be related with lack of facilities for detection and regulatory bodies that control the drug residues level in foods in the form of maximum residue limits (MRLs) **Kebede *et al.,* (2014).**

Effect of heat treatment:

People always were boiling milk as traditional method before consumed it , Milk processing for long-time boiling is better than short-time ultra-high temperature treatment in breaking the antibiotic residues in milk **Tian *et al.,* (2017).**

Table (4) showed the mean value of initial antibiotic residues in milk samples before heat treatment was 6.95± 1.36 ppb, 8.67± 2.42 ppb, and 8.68± 1.90 ppb for Amoxicillin, Ceftriaxone and Ciprofloxacin residues respectively. The antibiotic residues after heat treatment was a significant decreased to 1.04 ppb, 1.09 ppb and 6.33 ppb for Amoxicillin, Ceftriaxone and Ciprofloxacin residues respectively. The reduction percent of Amoxicillin, ceftriaxone and ciprofloxacin was 85%, 87.4% and 27%, respectively. Our results indicate thatβ -lactames antibiotics residues were heat labile, while fluoroquinolones were heat stable antibiotics.

The current results is nearly similar to that reported by **Roca *et al.*,(2010)**who mentioned that theheat treatment may have a minor role in reduction of quinolones residues in milk. Also **Roca *et al.*, (2011)** supported our finding that β-lactams were heat labile by boiling and their percentage reductionranged from 0.1-100 %.

Our results proven by results obtained by **Zorraquino *et al.*,(2008) and Traub and Leonhard, (1995)** who stated that classic sterilization (120°C for 20 min) and autoclaving at 121°C showed a high level of inactivation of penicillins and cephalosporins. while Ciprofloxacin heat-stable after autoclaving.

Incidance and concentrations of some heavy metals:

One of the main problems with metals is their ability to bio-accumulate in tissues of dairy animals and ultimately excrete in milk because of their non-biodegradable and persistent nature **Burger and Elbin (2015) and Meli *et al.*, (2015).**

Lead (pb):

Inspection of table (5) indicated that the incidence and mean concentration of lead in examined samples were 75.5% and 0.4555± 0.0828ppm, respectively. Lower results were obtained by **Ismail *et al.*,(2015) and Aamer *et al.*, (2016).** Higher results were recorded by **patra *et al.*, (2008) and Dawd *et al.*,(2012).**International Dairy Federation **IDF (1979)**, Codex Alimentarius International food standard **Codex (2018)** and Egyptian Standard **(ES 2010 No.7136)** adopted European Commission (**EC 2006 No. 1881**) for lead were stipulated by 0.02 ppm for each .43.33%of examined samples over permissible limit of all organizations. The higher content of Pb in milk may be attributed to industrial air pollution in this area especially all houses located at a traffic ways, the use of contaminated feed, and mineral supplements as well as water supply of bad hygienic quality **Santos *et al.*, (2015).** The high levels of heavy metals in some milk samples in this investigation may attribute to the high contamination of animal feed by such pollutants and also may be reached to the milk through handling procedures. The presence of Pb in milk samples could be due to various factors: transhumance along roads and/or motorways, fodder contamination, climatic factors, such as winds, and the use of pesticide compounds. One of the most important sources of lead contamination in milk is water, especially in more contaminated areas **(Codex Alimentarius Commission, 2003).**

Copper (Cu):

Copper naturally present as a micronutrient and its level in food was considered to reflect quality aspects rather than safety issues.

Inspection of (Table 5) indicated that, the incidence and mean concentration of copper in examined milk samples were 100% and 0.4179± 0.0508ppm, respectively. Lower results were obtained by **Tripathi *et al.,* (1999).** Higher records reported by **Bilandzˇic´*et al*., (2011) and Malhat *et al*., (2012)** .The low concentrations of Cu could be due to Zn contained in food that interferes with the copper absorption system, explaining the presence of low levels of this metal in milk **Doull's, (2000).**Possible contamination of milk with copper (Cu) can occur from animal feed, high copper content of water also fromits copper-based equipment **Mitchell, (1981).**

Zinc (ZN):

In the current study the incidence and mean concentration of Zinc in examined samples were 100 % and 2.971± 0.241ppm, respectively. Nearly similar results reported by **Qin *et al.,* (2009).** A possible source of contamination of zinc in milk is the use of metal cans and processing equipment **Jarrett, (1979).**

Chemical preservatives in milk samples:

Consumption of adulterated milk by consumers results in various health problems, and is a most important concern in the food industry **Singh *et al.,*(2010)**.Addition of any kind of preservatives to milk such as formalin, boric acid, salicylic acid and hydrogen peroxide is considered an illegal action as they have adverse effect on health. **(Azad and Ahmed 2016).** Inspection of (Table 6) indicated that 7.77 %(7/90) of the examined milk samples only adulterated by formalin. Other preservatives (Salicylic acid, Boric acid and Hydrogen peroxide could not be detected in any of examined milk samples. Also formalin could not detected in milk in previous study by **Makadiya and Pandey (2015).** Lower incidence of formalin in milk was recorded by **mansour *et al.,* (2012)**. Higher records were obtained by **Barham *et al.,* (2014)**. Boric acid and hydrogen peroxide could be detected by **Wahba and Korashy, (2006)** at percentage of 8.1 % and 3.8 %, respectively. These results could explain that formalin as a chemical inhibitory substance was a famous preservative for milk because it had the property of being in a liquid form.

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تقييم بعض المتبقيات الضارة في الالبان

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الملخص العربي

أجريت الدراسة الحالية في محافظة الإسماعيلية وشملت فحص90 عينة الألبان تم جمعها عشوائياً من الأسواق و الباعة الجائلين ومحلات بيع الألبان. وقد خضعت هذه العينات لاختبارات لتحديد تواجد متبقيات المضادات الحيوية باستخدام الاختبارات الميدانية المناعية والكروماتوغرافية السريعة فحص كروماتوغرافي مناعي قائم على الذهب (AuroFlow ™ Test Kits) وأوضحت الدراسة وجود متبقيات المضادات الحيوية البيتالاكتاماتbeta -Lactamsوالكينولون Quinolones في عينات الحليب الخام 42.2 ٪ و 21.1 ٪ ،على التوالي. وشفعت العينات بفحوصات تأكيدية وتحديدية باستخدام تحليل كروماتوجرافي سائل عالي الأداء (HPLC) وأظهرت النتائج أن32.2 % ،7.8 %,و21.1 ٪ من عينات اللبن التي تم تحليلها كانت ملوثة بمضادات الحيوية الأموكسيسيلين Amoxicillin،سيف ترياكسون Ceftriaxone وCiprofloxacin،على التوالي.وبدراسة تأثير الغليان على متبقيات المضادات الحيوية وجد مستوى الأموكسيسيلين وسيف ترياكسون وسيبروفلوكاسين فى عينات الألبان قبل عملية الغليان 8.67, 6.95 و 8.68 ميكروجرام / لترعلى التوالي. بينما كان مستوى متبقيات نفس المضادات الحيوية فى العينات بعد عملية الغليان.1.09,.1.04 و 6.33 ميكروجرام / لترعلى التوالي. وتم الكشف عن مستويات متبقيات المعادن والعناصر الثقيلة فيعينات الالبان وكان متوسط تركيز الرصاص , النحاس والزنك فى العينات 0.455 ,0.4179 و2.971مجم/لترعلى التوالى,.وقد أجريت اختبارات كيميائية لتحديد وجود الفورمالين، بيروكسيدالهيدروجين، حمض البوريك وحمض الساليسيليك. وكانت عينات الالبان خالية من الموادالحافظة الكيميائية الثلاثة في الحليب، في حين أن7.7 ٪ من العينات هي الأسوأ، لأنها تحتوي على الفورمالين.خلصت الدراسة إلى أن التلوث الكيميائي للحليب يشكل خطرا على صحة المستهلك. لذلك، يوصى باستخدام اختبارات ميدانية سريعة أثبتت فعاليتها في سرعة المتابعة والرصد المستمر للمتبقيات الضارة لتجنب وصولها إلى الغذاء مع ضرورة التوعية المستمرللمنتجين والمستهلكين عن التلوث الكيمائى للألبان.