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Determination the Presence of Antibiotics Residues in Raw Buffalo Milk in Assiut Governorate, Egypt

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NINETY samples of raw buffalo milk were collected randomly from markets in different localities in Assiut Governorate, Egypt. Samples were collected during the period from July 2022 to June 2023 from dairy farms, farmers' houses, and street vendors (thirty of each) to detect antibiotic residues in samples. Acidification test and Agar diffusion test were used in this study which has been applied in the European Community since 1 January 2002 (the European 91/180.CEE, EC Regulation Nr. 1664/2006), and described by the official European method for detecting antibiotic residues in milk (Commission Decision 91/180/EEC of 14 February 1991). The results show that the presence of antibiotics residues in 48 (53.33%) from a total of 90 examined raw milk samples and the frequency distribution in dairy farms, farmers' houses, and street vendors (30 each) examined samples based on acidification diffusion test were 28 (93.33%), 6 (20%) and 14 (46.66%) respectively and antibiotics residues by agar diffusion test were 46 (95.84%) from total 48 examined raw milk samples and the frequency distribution in examined samples were 27 (96.43%) & 6 (100%) &13 (92.86%) from 28 & 6 & 14, respectively. The families of antibiotics residues in the examined samples based on the Agar diffusion test were Penicillins, Tetracyclines Macrolides, Aminosides, and Sulfamides and the corresponding Antibiotics were (Penicillin G, Tetracycline), (Spiramycin, Erythromycin & Streptomicin) and (Trimethoprim, sulfamethoxazole) respectively. The high distribution frequency of contamination percentage with penicillin and /or tetracycline was 24 (52.17%) from a total of 46 examined samples and the frequency distribution were 14 (51.85%), 3 (50%) and 7 (53.85%) in other hand percentages of contamination with Macrolides and/or Aminosides were 12 (26.09%) from total 46 examined raw milk samples and the frequency distribution were where 5 (18.52%), 2 (33.33%) and 5 (38.46%) while the percentage of contamination with Sulfamides were 10 (21.74) from total 46 examined raw milk samples and the frequency distribution were 8 (29.63%), 1 (16.67%) and 1 (7.69%) from (27,6 and 13) examined positives raw milk samples of dairy farms, farmers houses, and street vendors respectively. The healthy importance, of antibiotic residues in raw milk and the methods of antibiotic control were discussed.

Keywords: Antibiotics, Assiut, Egypt, Raw milk, Residues.

Introduction

Milk and milk products considered one of the most important natural healthy food for human been at all ages of life which contain a complete formula of diet including fat (3.3-5.4%), proteins (3.0-3.9%), lactose (4.4-5.6%), vitamins as vitamin A, vitamin D, vitamin B12, and vitamin B2 and minerals as calcium, phosphorus, iodine, potassium, magnesium, zinc (Roy et al., 2020), so efforts had made to produce clean milk free from any contamination specially that come from treatment with drug as antibiotics. Antibiotics one of the most important drugs which used in Modern farms for prevention and treatment of many diseases, growth promoting agents (Barros et al., 2023) weight gain (Lees et al., 2021) and increase mik production (Bacanlı & Başaran, 2019) which may be secreted in milk at time of treatment or within the withdrawal time (period of time before all traces of the medication leaves the body (Stella et al., 2020; Vercelli et al., 2023). Presence

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of the antibiotics residue in the raw milk inhibit partially or completely starter microorganisms used in dairy products manufacturing (lactic acid bacteria) as *Streptococcus thermophillus*, *Lactobacillus bulgaricus* and *Lactococcus lactis* which cause loss of its function in manufacturing of dairy products (Maharjan et al., 2020; Ghidini et al., 2002) by precipitation of milk casein (protein), the development of aromas linked to proteolytic activity and lipolytic lactic strains which cause economic losses to dairy industries as cheese and yogurt (Rogister, 2002). On the other hand, the consumption of milk contaminated with antibiotics residue lead to appearance of (antibiotic-resistant pathogen strains) strains of microorganisms resistant that type of antibiotic which consummated with milk for long periods among human and animal populations worldwides (Vercelli et al., 2023), as meticillin-resistant *Staphylococcus aureus* and carbapenem-resistant Enterobacteriaceae. In addition to the risk of uncontrolled proliferation of gasifier's germs, insensitive to antibiotics, such as coliforms, *Bacillus*, *Clostridium* and *Proteus* Which lead to unresponsive to antibiotic treatment partially or completely (Vercelli et al., 2022). The antibiotics residues in milk can also affect consumer health which take the various type of antibiotics regularly for log time which may be cause effects the human gut flora composition) (Sachi et al., 2019; Treiber & Beranek-Knauer, 2021; Yazdanpanah et al., 2021) and hypersensitivity (Ezenduka et al., 2019). In addition to that the using of antibiotics as tetracycline for long time may cause digestive disorders, destruction of the liver, yellowing of the teeth and the tooth hyperplasia (Kang'ethe et al., 2015) and end by cancer, mutagenicity and toxicity. The most famous antibiotics used in treatment veterinary diseases in Egypt were pencilline, tetracycline, gentamicin, neomycin, sulfamethazine and chloramphenicol etc. Due to easily accessible of veterinary drugs to farmers and their distribution is not controlled by the government authorities lead to uncontrolled distribution and usage of antibiotics by public and unspecialized workers. That end by appearance of antibiotics residues of in milk and reach to consumers and not only, it constitutes a health risk but also a great economic risk (Almashhadany, 2021). So this study planned to determine and secure antibiotics residual in raw buffalo milk in dairy farms, farmers houses and street vendors (30 samples each) in different areas of Assiut Governorate, Egypt.

Materials and Methods

Collection of samples

Ninty random samples of raw bufflo milk were collected from markets in different localities in village near Assiut City famous by production a very large quantity of buffalo milk (Bani mor), Egypt. Samples were collected during the period from July 2022 to to June 2023 from dairy farms, farmers houses and street vendors (thirty of each) to detection of antibiotic residues in samples. Each sample of raw milk was put in an ice tank with thermometer at 4^0C. The samples were transferred to the laboratory to examination.

Raw milk samples

Scarding the first few drops of the collected raw buffalo milk samples then put every sample in in clean, dry, sterile screw capped bottles. Acidification test and Agar diffusion test were usedin this study which is applied in European Community since 1 January 2002 (the European 91/180.CEE, EC Regulation Nr. 1664/2006), and described by the official European method for detecting antibiotic residues in milk (Commission Decision 91/180/EEC of 14 February 1991) .

Acidification test

That test is the initial indicator for presence of antibiotic residues in examined milk samples or not. That test made by addition of milk sample in the nutrient agar containing a pH indicator and spores of *B. stearothermophillus* which good sensitivity to antibiotic residues specially penicillin. That causes a color change of the pH indicator (bromocresol purple) which turns from purple to yellow. All positive examined samples by acidification test followed by a Agar diffusion test.

Agar diffusion test

Muller-Hinton agar was previously melted at 100°C and cooled at 55°C before being poured into Petri dishes inoculated with 103 to 105 spores of *B. stearothermophillus, B. subtilis* and *B. megaterium*. After preparing a sterile paper disc, the latest were impregnated with milk samples and deposited on the agar surface. The Petri dishes were incubated at 55°C for *B. stearothermophillus* and 37°C for *B. subtilis* and *B. megaterium*. Incubation for 24h of, then measure the diameters of inhibition areas by using calipers.

Results and Discussion

Our study materials was represented by raw buffalo milk collected from dairy farms and farmers houses and street vendors. *B. stearothermophillus* used as test strain due to its sensitivity to antibiotics at high concentrations of antibiotics give small area of inhibition (Hilan & Chemali, 1998). The results in Table 1 show that the presence of antibiotics residues in 48 (53.33%) from total 90 examined raw milk samples and the frequency distribution in dairy farms, farmers houses and street vendors (30 each) examined samples based on acidification diffusion test were 28 (93.33%), 6 (20%) and 14 (46.66%), respectively. The results in Table 1 show that the presence of antibiotics residues in 48 (53.33%) from total 90 examined raw milk samples and the frequency distribution in dairy farms, farmers houses and street vendors (30 each) examined samples based on acidification diffusion test were 28 (93.33%), 6 (20%) and 14 (46.66%), respectively.

The data recorded in Table 2 appear that the antibiotics residues in present in 46 (95.84%) from total 48 examined raw milk samples and the frequency distribution in dairy farms, farmers houses and street vendors examined samples based on agar diffusion test were 27 (96.43%), 6 (100%) & 13 (92.86%) from 28, 6 & 14, respectively. These results are agree with those obtained by Navratilova (2008), Titouche et al. (2013) and Dabbagh Moghaddam et al. (2014). Lower results obtained by Azza (2006), Popa et al. (2010), Zolfaghari (2012), Elena et al. (2015), Leandro et al. (2018) and Almashhadany (2021).

Data recorded in Table 3 show that families of antibiotics residues in the examined in the raw buffalo milk examined samples based on Agar diffusion test were Penicillins, Tetracyclines Macrolides, Aminosides and Sulfamides and the corresponding Antibiotics were (Penicillin G, Tetracycline), (Spiramycin , Erythromycin &Streptomicin) and (Trimethoprim, sulfamethoxazole), respectively.

The results in Table 4 show that high contamination % by penicillin and tetracycline 24 (52.17%) from total 46 examined samples and the frequency distribution were 14 (51.85%), 3 (50%) and 7 (53.85%) in other hand percentage of contamination with Macrolides and/or Aminosides were 12(26.09%) from total 46 examined raw milk samples and the frequency distribution were where 5 (18.52%), 2 (33.33%) and 5 (38.46%), while percentage of contamination with Sulfamides were 10 (21.74) from total 46 examined raw milk samples and the frequency distribution were 8 (29.63%), 1 (16.67%) and 1 (7.69%) from (27, 6 and 13) examined positives raw milk samples of dairy farms, farmers houses and street vendors, respectively, out of (27, 6 and 13) examined positives raw milk samples. Nearly similar results obtained by TitoucheI et al. (2013) and Dabbagh Moghaddam et al. (2014).

Results of our study indicate that high contamination of raw buffalo milk examined samples by antibiotic residues that's may be due to uncontrolled uses of antibiotics in treatment of infectious diseases specially mastitis (Barros et al., 2023) on other hand using of veterinary drugs as growth promoters or as therapeutic remedies in wide range by farmers and dairy farms. Presence of that antibiotics residue in the raw milk inhibit partially or completely starter microorganisms used in dairy products manufacturing (lactic acid bacteria) as *Streptococcus thermophillus*, *Lactobacillus bulgaricus* and *Lactococcus lactis* which cause loss of its function in manufacturing of dairy products (Maharjan et al., 2020) by precipitation of milk casein (protein). The development of aromas linked to proteolytic activity and lipolytic lactic strains which cause economic losses to dairy industries as cheese and yogurt (Rogister et al., 2002).

The antibiotics residues in milk can also affect consumer health which take the various type of antibiotics regularly for log time which may be cause effects the human gut flora composition (Sachi et al., 2019; Treiber & Beranek-Knauer, 2021; Yazdanpanah et al., 2021) and hypersensitivity (Ezenduka et al., 2019).

In addition to that , appearance of strains of microorganisms (antibiotic-resistant pathogen strains) resist that type of antibiotic which consummated with milk for long periods among human and animal populations worldwide (Vercelli et al., 2023) as meticillin-resistant *Staphylococcus aureus* and carbapenem-resistant Enterobacteriaceae and may be cause proliferation of gasifier's germs, insensitive to antibiotics, such as coliforms, *Bacillus*, *Clostridium* and *Proteus* which lead to unresponsive to antibiotic treatment partially or completely (Vercelli et al., 2022) lading to uses of other antibiotic drugs more expensive in high-income countries and appear more morbidity and mortality in low-income and middle-income countries.

TABLE 1. Statically analytic result of antibiotics residues in the raw buffalo milk examined samples based on acidification test

Investigated		Negative samples		Positive samples	
Sample category	**No. of exam. samples**	**No**	**%**	**No**	**%**
dairy farms	30	2	6.67	28	93.33
Farmers house	30	24	80	6	20
street vendors	30	16	53.34	14	46.66
Total	90	42	46.67	48	53.33

TABLE 2. Statically analytic result of antibiotics residues in raw buffalo milk the examined samples based on agar diffusion test

Investigated		Negative samples		Positive samples	
Sample category	**No. of exam. samples**	**No**	**%**	**No**	**%**
dairy farms	28	1	3.57	27	96.43
Farmers house	6	0	0	6	100
street vendors	14	1	7.14	13	92.86
Total	48	2	4.16	46	95,84

TABLE 3. Families of antibiotics residues **in the in the raw buffalo milk examined samples based on *Agar diffusion test***

Test	Antibiotics families	Antibiotic
B. subtilis	Macrolides Aminosides	Spiramycin Erythromycin Streptomicin
B. megaterium	Sulfamides	Trimethoprim Sulfamethoxazole
B. Stearothermophillus	Penicillins Tetracyclines	Penicillin G Tetracycline

TABLE 4. Antibiotics residues families % in the in the raw buffalo milk examined samples

	Positive samples		No. and % of positive cases		
Sample category	**No**	**%**	**Penicillins Tetracyclines**	**Macrolides Aminosides**	**Sulfamides**
Dairy farms	27	96.43	14--51.85	5--18.52	8--29.63
Farmers houses	6	100	3--50	2--33.33	1--16.67
Street vendors	13	92.86	7--53.85	5--38.46	1--7.69
Total	46	95.84	24--52.17	12-26.09	10-21.74

Conclusions

Our study indicate that raw buffalo milk coming from different sources as dairy farms, farmers houses and street vendors in different areas of Assiut Governorate, Egypt were moderate to highly contaminated by antibiotics residues. Accessible of Veterinary drugs to farmers, and their distribution is not controlled by the government authorities. So firstly distribution of Veterinary drugs and antibiotics must be controlled by the government authorities and some recommendations must be taken in consideration to obtain clean milk free from antibiotic residues such as treated dairy animals must be isolated from production until complete recovery, rapid screening kits to detection of antibiotic residues in raw milk must be used as a routine examination by farmers and in the dairy factories. Must know all knowledge about the withdrawal time of any antibiotic used and not used milk until end of this time and periodical and regular examination of milk for antibiotic residues before consumption.

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الكشف عن مدى ونسبة تواجد بقايا المضادات الحيوية في حليب الجاموس الخام في محافظة أسيوط . مصر

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مستشفيات جامعة اسيوط- جامعة اسيوط- اسيوط- مصر.

تم جمع عدد 90 عينة عشوائية من الحليب الخام من مزارع الألبان ومنازل المزارعين والباعة الجائلين (30 عينة لكل منهم) من محافظة أسيوط، مصر. فى الفترة من مايو 2022 إلى أبريل 2023 وتم إجراء الكشف عن بقايا المضادات الحيوية في العينات وفقًا للطريقة الأوروبية الرسمية للكشف عن بقايا المضادات الحيوية في الحليب (قرار المفوضية EEC / 91/180 بتاريخ 14 فبراير 1991)، والذي يتم تطبيقه فى أوربا منذ 1 يناير 2002 وتم استخدام اختباري acidification test and agar diffusion test وأوضحت النتائج أن بقايا المضادات الحيوية توجد في 48 عينة بنسبة (53.33٪) من إجمالي 90 عينة تم فحصها من الحليب الخام في مزارع الألبان ومنازل المزارعين والباعة الجائلين (30 عينة لكل منها) بناءً على اختبارacidification test وكان وكان التوزيع التكراري في العينات التي تم فحصها 28 (93.33) ٪) و 6 (20٪) و 14 (46.66٪) على التوالي، وبلغت بقايا المضادات الحيوية عن طريق اختبار acidification test 46 (95.84٪) من إجمالي 48 عينة لبن خام تم فحصها، وكان التوزيع التكراري في العينات التي تم فحصها 27 (96.43٪) و 6 (100٪) و 13 (92.86٪) من اجمالى 28 و 6 و 14 على التوالي. وكانت عائلات بقايا المضادات الحيوية في العينات التي تم فحصها بناءً على اختبارacidification test هي البنسلين، التتراسيكلين ماكرولايد، الأمينوسيدات والسلفاميدات والمضادات الحيوية المقابلة هي (البنسلين جي، التتراسيكلين)، (سبيرامايسين، إريثروميسين وستربتوميسين) و (تريميثوبريم ، سلفاميثوكسازول) على التوالي. كانت النسبة العالية للتلوث بالبنسلين و / أو التتراسيكلين 24 (52.17٪) من إجمالي 46 عينة تم فحصها والتوزيع التكراري 14 (51.85٪) ، 3 (50٪) و 7 (53.85٪) من ناحية أخرى نسبة التلوث بالجرثومة. كانت الماكروليدات و / أو الأمينوسيدات 12 (26.09٪) من إجمالي 46 عينة من الحليب الخام التي تم فحصها وكان التوزيع التكراري 5 (18.52٪) و 2 (33.33٪) و 5 (38.46٪) بينما كانت نسبة التلوث بمركبات السلفاميد 10 (21.74) من إجمالي 46 عينة من اللبن الخام المفحوصة وكان التوزيع التكراري 8 (29.63٪) ، 1 (16.67٪) و 1 (7.69٪) من (27.6 و 13) في عينات اللبن الخام التي تم فحصها من مزارع الألبان وبيوت المزارعين. والباعة الجائلين على التوالي.و تمت مناقشة الأهمية الصحية لبقايا المضادات الحيوية في اللبن الخام وطرق التحكم فيها.