Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1154 - 1171
Received 05 March 2021; Accepted 01 April 2021.

Assessment of Antibiotic Residues in Cow's Milk Collected from the Different Regions of Libya

Salem Abdulsalàm Ali Dabi[1], Adel Abdelkhalek Sayed Ahmed[2] and Mohammed El-Sherbini[3].
[1]Food Sciences and Technology Department Faculty of Engineering and Technical Sciences. University of Sabha Libya, [2]Food Hygiene and Control Department Faculty of Veterinary Medicine. Mansoura University 35516 Egypt, [3]Food Hygiene and Control Department Faculty of Veterinary Medicine. Mansoura University 35516 Egypt.
Salemdebey@yahoo.com; Adel.Abdelkhalek@buc.edu.eg

Abstract: The current study was applied on 200 cows milk samples, which collected randomly from different cities in Libyan, were included 53 cow raw milk, 27 pasteurized milk and 120 sterilized milk. Examined qualitative by using Delvo test SP NT, the results revealed that antibiotic residues were 75.47% and 48.15% in cow raw milk and pasteurized milk, respectively. And all the sterilized milk tested were 100% negative for a test. High liquid performance chromatography (HPLC) were used as confirmatory method for identification of Beta-lactam residues in 14 raw milk positive with Delvo test SP NT. The detected of Ampicillin, Amoxicillin, Tetracycline and Oxytetracycline residues in cow raw milk were 35.71%, 71.42%, 85.71% and 78.57%, respectively. Concentration limits a mean value 4.88±1.02µg/kg, 12.22±6.18µg/kg, 86.24±56.60µg/kg and 108.40±54.15µg/kg for Ampicillin, Amoxicillin, Tetracycline and Oxytetracycline residues, respectively. In raw milk. Confirmed, Amoxicillin, Tetracycline and oxytetracycline residues were 2/6 (33.33%), 5/6 (83.33%) 5/6 (83.33%) and 6/6 (100%) In pasteurized milk, respectively. With concentration limits the mean value 3.45±0.25µg/ kg Ampicillin, 14.06±3.97µg/kg 112.50±89.04µg/kg and 124.30±81.74µg/kg, for Ampicillin, Amoxicillin, Tetracycline and Oxytetracycline residues in pasteurized milk, respectively. In this study examined 20 sterilized milk, showed that antibiotic residues were not detected by used high-performance liquid chromatography.

Keywords | Dairy, Antimicrobials, Penicillin, Drugs, Allergic.

INTRODUCTION:

There are various types of drugs, particularly antibiotics, which are utilized for treatment of diseases and infections in dairy farms. In veterinary medicine, antibiotics are commonly utilized to treat bacterial infections (mainly mastitis) and to cure secondary bacterial infection in cases of viral infections. Also, antibiotics are utilized in prophylactic doses to prevent diseases and enhancing feed

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1154 - 1171
Received 05 March 2021; Accepted 01 April 2021.

efficiency.

Beta-Lactam group is one of the mainly utilized antibiotic groups which comprises a group of antibiotics that have same structure unit (Beta-Lactam ring) such as Penicillin, Amoxicillin, Monobactams, Carbapenems, Cephalosporins, Cephamycins, and betalactamase inhibitors ***(Yıldız, 2008)***.

Beta-Lactam antibiotics share a similar mechanism of action through inhibiting synthesis of cell wall of bacteria ***(Novak et al., 2000)***.

Beta-Lactam antibiotics are widely utilized because of their impact on both Gram positive and negative bacteria so that in dairy industry many problems are caused by their residues ***(Gustavsson et al., 2002; Jacoby and Munoz-Price, 2005)***.

Deficient farmers' knowledge regarding withdrawal time of antimicrobials, excess antibiotics usage without appropriate treatment records in dairy farms, non-observance of each antibiotic withdrawal time, higher dosages and adding such antimicrobials as preservatives to milk result in raw milk contamination with residues of Beta-Lactam ***(Ruegg, 2009)***.

There are possible health hazard for the consumers results from contaminated milk by residues of Beta-Lactam such as allergic reactions in hypersensitive persons, drug resistance, growth of resistant bacteria, mutagenic and carcinogenicity effects result from prolonged exposure to these residues. Even with low concentrations of Beta-Lactam antibiotics residues particularly penicillin, approximately 10% of people are hypersensitive ***(Khaskheli et al., 2008)***.

In dairy industry, there are great economic losses because of Beta-Lactam residues' inhibitory impact on starter cultures utilized in yoghurt, butter, cheeses, and other products as it decreases acid formation, prolongs milk curdling time and cause inadequate ripening of cheese and thus, has an effect on dairy products' quality ***(Rinken and Riik, 2006; Božo and Anđel, 2008)***.

Maximum residue limitations of Beta-Lactam antibiotics such as Penicillin G 4µg/L, Amoxicillin 4µg/L, Ampicillin 4µg/L, Dicloxacillin 30µg/L, Cepharin 60 µg/L and Cephalexin 100µg/L were reported in the European Union Regulation ***(Movassagh and Karami, 2011)***

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1154 - 1171
Received 05 March 2021; Accepted 01 April 2021.

AIM OF THE WORK:

The current work was conducted for investigation of the following issues:

1-Qualitative determination of antibiotic residues in 200 randomly collected and different cow milk samples by utilizing test Delvo SP NT

2-Quantitative determination of Beta-Lactam residues in samples with positive results with Delvo test SP NT utilizing High performance liquid chromatography (HPLC).

3-Highliting the public health hazards and economic importance of Beta-Lactam residues in cow milk.

MATERIALS AND METHODS:

1-Collection of milk samples *(American Public Health,1985)*:

All milk samples can be collected from Libya.

The laboratory work was performed in the Department of Food Hygiene of control, Faculty. Veterinary. Medicine. Mansoura. University. Egypt.

The period between May 2019 to October 2020, all examined milk samples were subjected to qualitative and quantitative detection of Beta- lactam antibiotic residues and other.

A total of 200 cow milk samples from various sources were obtained from various cities in Libyan (Tripoli, Tajoura, Janzur, ALzawiyah, Subrata, Misrata, Sabha, Brak), The samples from different private of markets and dairy shops, were included 53 cow's raw milk samples, Each raw milk sample was received in 300 ml sterile capped plastic jar and stored at (-20°C), 27 Pasteurized milk samples, were atoned from different private of markets (capacity one liter packet), and stored at (-20°C), 120 Sterilized milk samples from different private of markets capacity one liter packet, production different countries Companies were showed Table, with attention to production dates, expiry dates, serial numbers of all packs.

And stored at room temperature (25°C), then transferred raw milk samples and Pasteurized milk samples in an ice box at (3±1°C) to milk hygiene and control department,

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1154 - 1171
Received 05 March 2021; Accepted 01 April 2021.

Mansoura University Egypt, collected samples were kept at (3±1°C), tile preparation to Detection of antibiotics.

2-Preparation of milk samples for analyses:

(A)- determination of sample rawness Storch test ***(Quehl, 1977)***:

Ten ml of sample was put in a test tube, Two drops of hydrogen peroxide solution, 2 drops of aqueous solution of paraphenylene diamine (2%) were added then mixing was performed. Sample rawness was indicated by an appearance of dark bluish coloration.

(B) Detection of efficacy of pasteurization:

The original packs of pasteurized milk samples were collected and Transferred to the laboratory without delay, and subjected to lactognost phosphatase test ***(Collins-Thompson et al., 1988)***.

Delvotest SP-NT: Delvotest SP-NT ampoules as well as block heater were obtained (Netherland).

3- Quantitative determination of Beta-Lactam residue in positive samples by HPLC:

Only certain number of presumptive positive samples with Delvotest SP NT was transported using an ice box at (3±1°C) to Animal Health Research Institute in Giza, Egypt to identify Beta-Lactams residue and their levels in milk samples utilizing HPLC.

A- Milk sample preparation: Beta lactams determination (***Khaskheli et al., 2008)***

Five milliliters milk underwent mixing with 400µl of 10% aquous solution of acetic acid in a centrifuge tube. Then, agitation was performed for 60 sec by vortex. This was followed centrifugation at 3500 rpm for ten min at 4°C. Clear supematant was filtered utilizing 0.45µm filter and filtrate was poured in to a plastic vial and 5µl of it underwent injection in HPLC.

B- **Detection of Amoxicillin and Ampicillin as described by *Kuo et al. (2009)***

C- Tetracycline determination (*Abbasi et al., 2011)*

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1154 - 1171
Received 05 March 2021; Accepted 01 April 2021.

In brief, 15 ml milk underwent mixing in a 50 ml tube with 25ml Mellvaine buffer solution. This was followed by agitation of solution utilizing vortex for 60 sec. centrifugation was performed at 10,000 rpm for 12 minutes at 4°C. Supernatant was utilized in SPE cartridges which were treated by 3ml methyl alcohol at a flow rate not exceeding 3ml/minute. After that, it was rinsed by 2ml DI water. Extraction was performed through pouring 25ml of supernatant in SPE at a flow rate 5ml/minute then cartridge was eluted by methyl alcohol solon 5%. Elution was performed using 2ml methyl alcohol at rate 4ml/minute. Then samples were dried, diluted in 1ml of mobile phase and then filtrated to be injected in HPLC.

D- Quantitative detection of oxytetracycline according to *Cinquina et al. (2003)*.

RESULTS:

Table 1: Qualitative determination of antibiotic residues in examined samples used Delvo test SP NT.

Type of samples	No. of samples	No. of Negative samples	No. of positive samples	Percentage of positive samples%
Cows raw milk	53	13	40	75.47
Pasteurized milk	27	14	13	48.15
Sterilized milk	120	120	0	0
Total	200	147	53	26.50

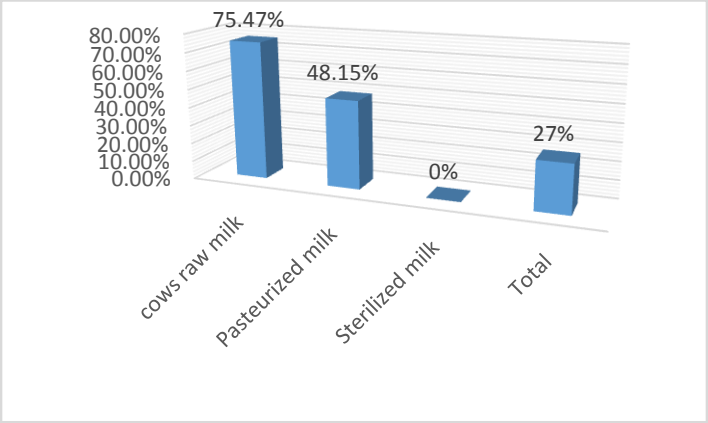


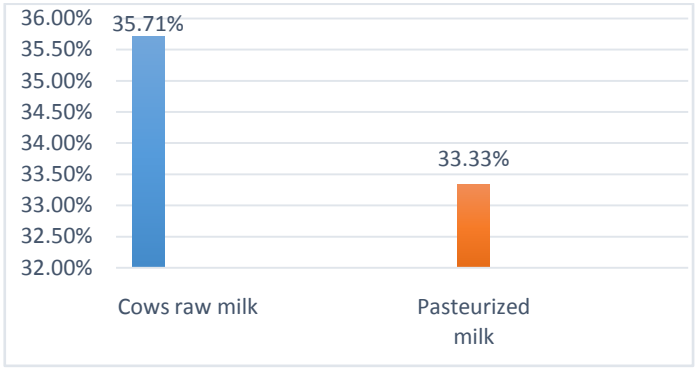
Figure 1: Qualitative determination of antibiotic residues in examined samples used Delvo test SP NT.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1154 - 1171
Received 05 March 2021; Accepted 01 April 2021.

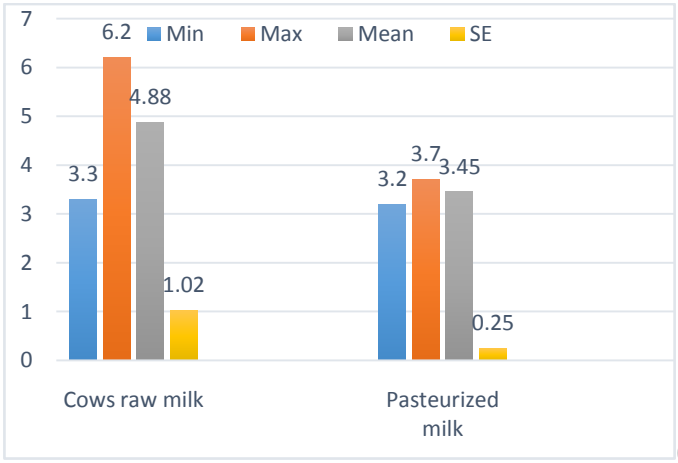
Table 2: Quantitative determination of Beta-Lactam Ampicillin residues positive Delvo test SP NT milk samples used HPLC.

Type of samples	No. of samples	N0. Of positive samples (HPLC)	%	Concentration of Ampicillin µg/kg			
				Min	Max	Mean	SE
Cows raw milk	14	5	35.71	3.30	6.20	4.88	1.02
Pasteurized Milk	6	2	33 .33	3.20	3.70	3.45	0.25
Sterilized milk	20	0	0	0	0	0	0

*Maximum residue limits (MRLs) Correspond to Codex Alimentarius Committee (CAC, 2018).



(A)



(B)

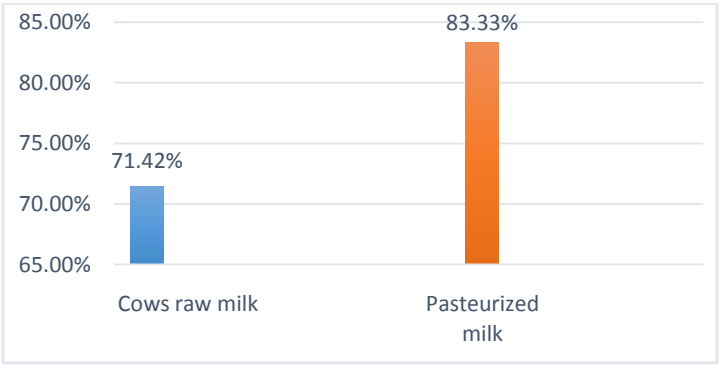
Figure 2 (A and B): Quantitative detection of Beta-Lactam Ampicillin µg/kg **residues positive Delvo test SP NT.**

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1154 - 1171
Received 05 March 2021; Accepted 01 April 2021.

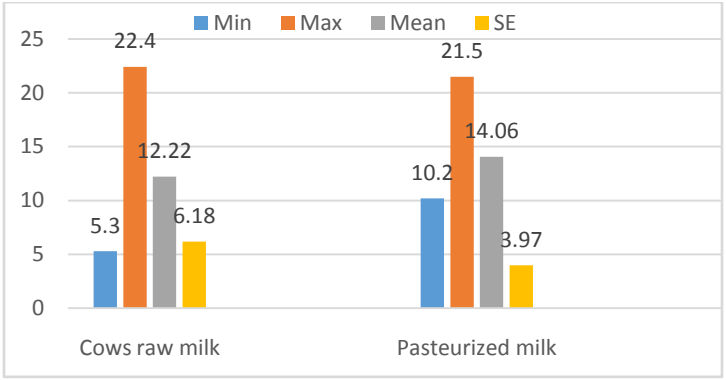
Table 3: Quantitative detection of Beta-Lactam Amoxicillin residues in positive Delvo test SP NT milk samples used HPLC.

Type of samples	No. of samples	N0. positive samples HPLC	%	Concentration of Amoxicillin µg/kg			
				Min	Max	Mean	SE
Cows raw milk	14	10	71.42	5.30	22.40	12.22	6.18
Pasteurized milk	6	5	83.33	10.20	21.50	14.06	3.97
Sterilized milk	20	0	0	0	0	0	0

*Maximum residue limits (MRLs) Correspond to Codex Alimentarius Committee (CAC, 2018).



(A)



(B)

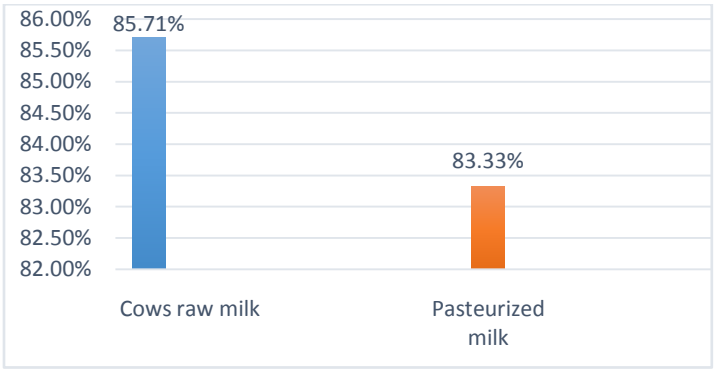
Figure 3 (A and B): Quantitative detection of Beta-Lactam Amoxicillin residues in positive Delvo test SP NT milk samples used HPLC.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1154 - 1171
Received 05 March 2021; Accepted 01 April 2021.

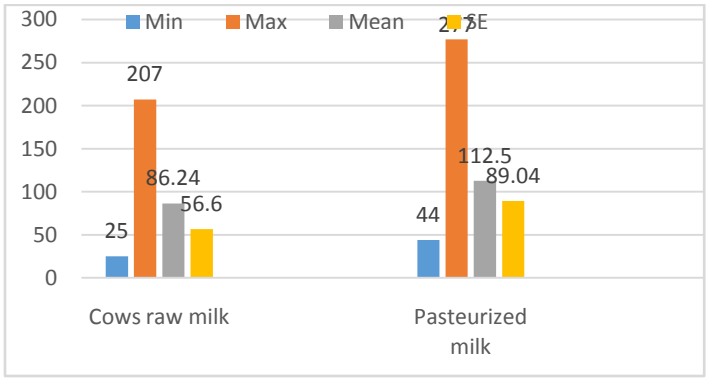
Table 4: Judgment the Concentration limits of Tetracycline residues in Samples used HPLC according to standard.

Type of samples	No. of samples	N0.of positive samples (HPLC)	%	Concentration of Tetracycline µg/kg			
				Min	Max	Mean	SE
Cows raw milk	14	12	85.71	25	207	86.24	56.60
Pasteurized milk	6	5	83.33	44	277	112.50	89.04
Sterilized milk	20	0	0	0	0	0	0

*Maximum residue limits (MRLs) Correspond to Codex Alimentarius Committee (CAC, 2018).



(A)



(B)

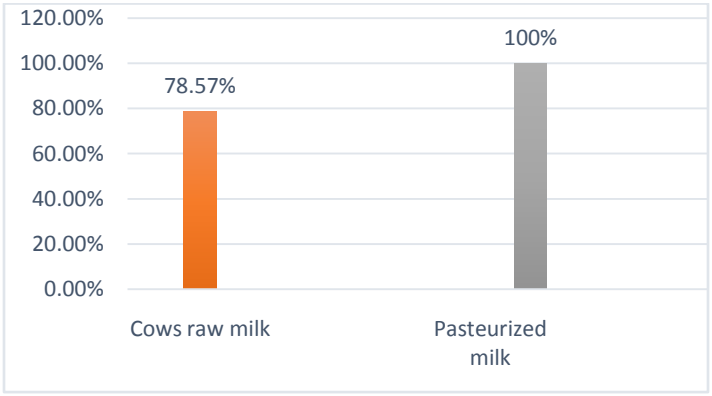
Figure 4 (A and B): Judgment the Co ncentration limits of Tetracycline residues in milk Samples used HPLC according to standard.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1154 - 1171
Received 05 March 2021; Accepted 01 April 2021.

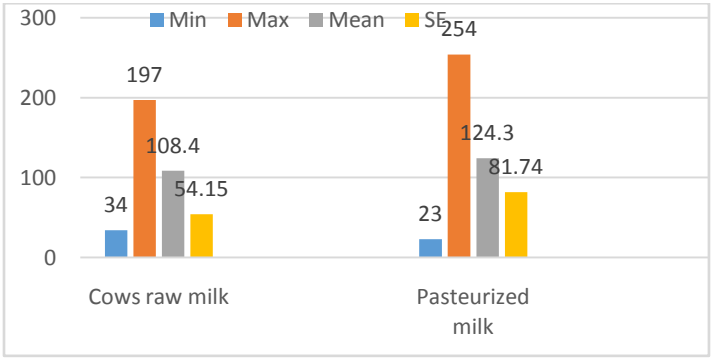
Table 5: Judgment the Concentration limits of oxytetracyclin residues in milk Samples used HPLC according to standard.

Type of samples	No. of samples	N0. of positive samples (HPLC)	%	Concentration of Oxytetracyclin µg/kg			
				min	max	Mean	SE
Cows raw milk	14	11	78.57	34	197	108.4	54.15
Pasteurized milk	6	6	100	23	254	124.3	81.74
Sterilized milk	20	0	0	0	0	0	0

*Maximum residue limits (MRLs) Correspond to Codex Alimentarius Committee (CAC, 2018).



(A)



(B)

Figure 5 (A and B): Judgment the Concentration limits of oxytetracycllin residues in milk Samples used HPLC according to standard.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1154 - 1171
Received 05 March 2021; Accepted 01 April 2021.

DISCUSSION:

A total of 200 milk samples (53 raw cow milk, 27 pasteurized milk and 120 sterilized milk) were collected from Libya. The samples were subjected to Delvo test for qualitative detection antibiotic residues in the laboratory of food Hygiene and Control Department, Fac. Vet. Med. Mansoura Univ, Egypt.

The results obtained demonstrated that the detectable antibiotic residues percentages were higher in cow raw milk samples 40 out of 53 (75.47) (Table 1 and Figure 1). The high levels of biological contamination of raw milk could be attributed administer antibiotic as prophylactic and therapeutic uses in lactating diseased animals, as no knowledge about withdrawal period of milk with of antibiotic residues. These results of study were nearly similar percentages antibiotic residues recorded by ***Sulejmani and Shehi (2012); Gallina et al. (2013)***. Higher percentages antibiotic residues detailed by ***Kress et al. (2007); Fejzic et al. (2014)***.

Delvo test SP NT detected antibiotic residues in 13/27 (48.15%) pasteurized milk samples. This finding nearly similar percentage antibiotic residues recorded by ***Tolentino et al. (2005)***. Higher percentage positive visualized by ***Ramírez et al. (2001)***.

Outlined qualitative detection of antibiotic residues in sterilized milk samples used Delvo test SP NT. The results obtained revealed that all examined sterilized milk samples undetectable antibiotic residues. This finding was about similar those recorded by ***Folly and Machado (2001); Movassagh and Karami (2011); Rama (2011); Kunda et al. (2015)***. The current result of study those declared by ***Mirlohi et al. (2013)*** who recorded no significant difference between contamination rate of pasteurized samples with antibiotic residues.

HPLC analysis was carried out of identification and confirmation of Ampicillin and Amoxicillin antibiotic residues. As the most beta lactam antibiotic used in the veterinary field.

(Table 2 and Figure 2 A, B) illustrated the quantitative detection of Ampicillin residues in examined milk samples. Five out of 14 (35.71%) confirmed Ampicillin residues in cow raw milk. The detection levels ranged from 3.3 – 6.2µg/kg and the mean value was 4.88±1.02 µg/kg. Two out of 6 (33%) pasteurized milk samples confirmed Ampicillin, the concentration levels was ranged from 3.2 – 3.7 with mean 3.45±0.25 µg/kg. All sterilized milk samples proved to be

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1154 - 1171
Received 05 March 2021; Accepted 01 April 2021.

Ampicillin free (undetectable). The result of this study was correspond to codex Alimentarius commission (CAC, 2018) (MRL, 4µg/kg). The results obtained cleared that only 4 cow raw milk samples exceed MRL of standard. In previous study, ***Brito and Junqueira (2006)*** whom recorded Ampicillin residues were recovered from 60% of milk samples with detection limits 4 µg/L.

In (Table 2 and Figure 2 A, B) confirmed Ampicillin residues were not detect in all sterilized milk samples. ***Rama (2011)*** recorded 2/69 UHT milk samples were Beta lactam residues. In Iran, ***Movassagh and Karami (2011)*** recorded 7 out of 150 (4.66%) UHT milk samples contained Beta lactam residues.

(Table 3 and Figure 3 A, B): demonstrated the identification and concentration limits of Amoxicillin in presumptive positive Delvo test SP NT milk samples used HPLC. Percentage of Amoxicillin residues in cow raw milk samples was 71.42% (10/14) of which concentration limits, Min., Max. and the mean were 5.30 , 22.4 and 12.22±6.18µg/kg, respectively. Compared to standard 10 raw milk samples were exceed MRL. ***Khaskheli et al. (2008)*** investigated 137 market milk samples, collected from Hyderabad city, Latifabad and Qasinabad for confirmation the concentration limits of Amoxicillin residues used HPLC. The results obtained concluded that Amoxicillin residues was contaminated 56% examined samples. The mean concentration of Amoxicillin residues were 1 – 190µg/L. In Faisalabad city, Pakistan. ***Irum et al. (2014)*** whom recorded that 90% of examined market milk samples were contained Amoxicillin residues with concentration ranged from 28 – 46µg/kg. All samples were above standard limits. In Chittagom, ***Chowdhury et al. (2015)*** used HPLC for determination concentration of Amoxicillin residues. They found that Amoxicillin residues was detected in 14%, 38% of raw local and commercial milk samples, respectively. With concentration limits 9.84 and 56.16 µg/L, respectively. The mean concentration limits of Amoxicillin in market milk samples was 26.15 µg/kg ***(Priyanka et al., 2019)***. The biological contamination rate of pasteurized milk with Amoxicillin residues was 83.33% (5/6) with mean concentration limit was 14.6±3.97 µg/kg. (Table 3). ***Holstege et al. (2002)*** used HPLC for detection level of Amoxicillin in pasteurized milk They reported that the level was 0.1 ng/ml. According to codex AlimentArius commission standard ***(Commision, 2011)*** only 10 and 5 cow raw milk and pasteurized milk samples,

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1154 - 1171
Received 05 March 2021; Accepted 01 April 2021.

respectively over MRL (Table 3). The results of this study indicated all examined sterilized milk samples proved to be undetectable Amoxicillin residues.

Tetracycline was widely used due to their broad spectrum activity against pathogenic microorganisms, Low toxicity and low cost. So they were applied for treatment and prophylaxis of certain infectious diseases of dairy animals ***(Prescott et al., 2000)***.

(Table 4 and Figure 4 A, B) declared that quantitative estimation of tetracycline residues in milk and its Judgment in compliance with CAC used HPLC. Identification and confirmation tetracycline antibiotic residues in presumptive positive milk samples was 85.71% and 83.33% of cow raw milk and pasteurized milk, respectively. All sterilized milk samples was undetectable tetracycline residues. Contamination of raw cow milk by tetracycline was 12/14 (85.71%). The detection limits of tetracycline residues in cow raw milk samples was Min. 25µg/kg, Max. 207µg/kg and the mean 86.24±56.60µg/kg. Four tetracycline detectable cow raw milk samples were above codex Alimentarius commission (CAC, 208). Certain number comprehensive researches on occurrence of tetracycline in milk was done worldwide. Lower incidence were reported by ***Darwish et al. (2013)*** whom investigated milk samples for quantitative detection of tetracycline. They found that tetracycline were present in 41% of examined samples. ***Tempini et al. (2018)*** recorded 4/25 (16%) samples contamination of tetracycline residues. Additionally ***Mottaghiyanpour et al. (2018)*** serggested only 1% tetracycline residues in raw milk. On the other hand higher level of contamination was detected by ***Kurjogi et al. (2019)*** who stated that the concentration of tetracycline residues in cow raw milk was 5460 µg/kg. ***Patel et al. (2020)*** whom found The prevalence of tetracycline residues in bovine milk samples was 98.75% with a mean value 667.09µg/kg. The detection limit of tetracycline residues would likely exceed CAC standard. Its concentration was greater 8 Times Then standard, beside ***Patel et al. (2020)*** observed 98.15% of samples were contamination with tetracycline residues the mean was 667.09µg/kg. The concentration limits of tetracycline residues in pasteurized milk samples were ranged from 44 – 277µg/kg and a mean 112.50±89.04µg/kg. Two out of six pasteurized milk samples heavy biological contaminated with tetracycline residues over MRL.

(Table 5 and Figure 5 A, B): showed the percentage and concentration limits of oxtetracycline residues used HPLC correspond to codex Alimentarius commission standard, 2018. The detection of oxytetracycline residues milk samples 6/6 (100%) for owed by cow raw

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1154 - 1171
Received 05 March 2021; Accepted 01 April 2021.

milk 11/14 (78.57%) and undetectable oxytetracycline residues in all examined sterilized milk samples. The concentration limits were varied from 34 – 197 with mean 108.40±54.15 µg/kg in cow milk samples. The detection limits of oxytetracycline residues in pasteurized milk samples (Table 5 and Figure 5 A, B). The min. detectable concentration was 23µg/kg, The max. detectable concentration was 254µg/kg, with mean concentration 124.30±81.74µg/kg. The number of pasteurized milk samples heavy contaminated Oxytetracycline over MRL standard (100µg/kg) was 3 samples (Table 5). Higher incidence of Oxytetracycline residues obtained by ***Beltrán et al. (2013)*** who observed Oxytetracycline residues in all examined ovine milk. While lower incidence were obtained by ***Abbasi et al. (2011)*** who reported that 57.1% 0f raw milk samples were contaminated with Oxytetracycline residues used HPLC. In addition to ***Tempini et al. (2018)*** level 0f contamination was 16% and ***Makarem et al. (2020)*** quantitative detection Oxytetraycline residues in market raw milk samples collected from Alexandria. Governorate, Egypt. By using HPLC. They revealed that the rate of incidence was 30% with a mean value 97.9±28.1µg/kg. On other hand ***Oruç (2005)*** whom recorded that none of examined cow milk samples contained Oxytetracycline in Bursa, Turkey.

ECONOMIC STAND POINT: Their adverse effect on dairy product processors as soiled milk with high concentration of Beta-lactam residues would generated adverse effect on productivity of dairy products manufacture as inadequate ripening of chees ***Cámara et al. (2013)*** and inhibition of starter culture used in dairy products especially fermented products ***Phillips et al. (2004)*** lead to great economic losses.

PUBLIC HEALTH HAZARDS: The frequent use and misuse of antibiotic lead to the presence of antibiotic with high levels resulted in public health hazard as allergic reaction (anaphylactic, shock, skin rash, hiver an asthma), development of resistant m.os, elimination of carcinogenic effect on human through consumption of contaminated milk and its products by Beta-lactam residues ***(Tajick and Shohreh, 2006; Karraouan et al., 2009; Chowdhury et al., 2015)***.

CONCLUSION:

Stops should be applied to avoid contamination of milk and dairy products with Beta–lactam residues:

1- Good observation of withdrawal period of each residues.

2- In discriminate use of antibiotic not allow.

3- Good registered system for treated animals.

4- Frequent use low doses result in development of resistant strains of m.os failed to be treated.

5- Heat treatment during processing of products did not eliminate residues in the products.

6- Education farmers about adverse effect of Beta-lactam residues on dairy products processors. And the detection of B-lactam residues in milk has to be performed in farms for avoiding more economic loss related to Transportation process, and that related to loss of milk itself.

7- Detect B-lactam residues in different stages of dairy products manufacture.

REFERENCES:

[1]. **Abbasi, M. M., Babaei, H. & Ansarin, M. (2011).** Simultaneous determination of tetracyclines residues in bovine milk samples by solid phase extraction and HPLC-FL method. *Advanced Pharmaceutical Bulletin,* 1: (1), 34.

[2]. •**American Public Health, A.** APHA (1985): Standard methods for the examination of dairy products. American Public Health Association, New York.

[3]. •**Beltrán, M. C., Romero, T., Althaus, R. L. & Molina, M. P. (2013).** Evaluation of the Charm maximum residue limit β-lactam and tetracycline test for the detection of antibiotics in ewe and goat milk. *Journal of Dairy Science,* 96: (5), 2737-2745.

[4]. •**Božo, J. & Anđel, S. (2008).** Safety and fermentability of dairy products. *Food and Feed research,* 35: (2), 77-80.

[5]. •**Brito, R. B. d. & Junqueira, R. G. (2006).** Determination of Beta-Lactam residues in milk by high performance liquid chromatography. *Brazilian Archives of Biology and Technology,* 49: (spe), 41-46.

[6]. •**Cámara, M., Gallego-Picó, A., Garcinuño, R. M., Fernández-Hernando, P., Durand-Alegría, J. S. & Sánchez, P. J. (2013).** An HPLC-DAD method for the simultaneous determination of nine β-lactam antibiotics in ewe milk. *Food Chemistry,* 141: (2), 829-834.

[7]. •**Chowdhury, S., Hassan, M. M., Alam, M., Sattar, S., Bari, M. S., Saifuddin, A. K. M., *et***

al. **(2015).** Antibiotic residues in milk and eggs of commercial and local farms at Chittagong, Bangladesh. *Veterinary World,* 8: (4), 467-471.

[8]. **•Cinquina, A. L., Longo, F., Anastasi, G., Giannetti, L. & Cozzani, R. (2003).** Validation of a high-performance liquid chromatography method for the determination of oxytetracycline, tetracycline, chlortetracycline and doxycycline in bovine milk and muscle. *Journal of Chromatography A,* 987: (1-2), 227-233.

[9]. **•Collins-Thompson, D. L., Wood, D. S. & Thomson, I. Q. (1988).** Detection of Antibiotic Residues in Consumer Milk Supplies in North America using the Charm Test II Procedure. *Journal of Food Protection,* 51: (8), 632-633.

[10]. **•Commision, C. A. (2011).** Maximum Residues Limits for Veterinary Drugs In Food. CAC.

[11]. **•Darwish, W. S., Eldaly, E. A., El-Abbasy, M. T., Ikenaka, Y., Nakayama, S. & Ishizuka, M. (2013).** Antibiotic residues in food: the African scenario. *Japanese Journal of Veterinary Research,* 61: (Supplement), S13-S22.

[12]. **•Fejzic, N., Begagic, M., Šerić-Haračić, S. & Smajlovic, M. (2014).** Beta lactam antibiotics residues in cow's milk: comparison of efficacy of three screening tests used in Bosnia and Herzegovina. *Bosnian Journal of Basic Medical Sciences,* 14: (3), 155.

[13]. **•Folly, M. M. & Machado, S. d. C. A. (2001).**

a. Determination of antibiotic residues,

[14]. using methods of microbial inhibition, enzymatic and immuno-Assays in pasteurized milk marketed in the northern region *of the state of Rio de Janeiro, Brazil. Rural Science,* 31: (1), 95-98.

[15]. **•Gallina, G., Rama, A., Lucatello, L., Benetti, C., Bajraktari, D., Uka, K., *et al.* (2013).** Aflatoxin M1 Contamination and Antibacterial Residues in Milk in Kosovo. *Trends in Veterinary Sciences.* Springer Berlin Heidelberg. 109-113.

[16]. **•Ghanavi, Z., Mollayi, S. & Eslami, Z. (2013).** Comparison Between the Amount of Penicillin G Residue in Raw and Pasteurized Milk in Iran. *Jundishapur Journal of Microbiology,* 6: (7).

[17]. **•Gustavsson, E., Bjurling, P. & Sternesjö, Å. (2002).** Biosensor analysis of penicillin G in milk based on the inhibition of carboxypeptidase activity. *Analytica Chimica Acta,* 468: (1), 153-159.

[18]. **•Holstege, D. M., Puschner, B., Whitehead, G. & Galey, F. D. (2002).** Screening and Mass Spectral Confirmation of β-Lactam Antibiotic Residues in Milk Using LC-MS/MS. *Journal of*

Agricultural and Food Chemistry, 50: (2), 406-411.

[19]. **•Irum, A., Saeed, H., Shafaqat, A., Farid, M., Parveen, R., Maqbool, U., *et al.* (2014).** Estimation of amoxicillin residues in commercial meat and milk samples. *Journal of Microbiology, Biotechnology and Food Sciences,* 4: (1), 48-50.

[20]. **•Jacoby, G. A. & Munoz-Price, L. S. (2005).** The New β-Lactamases. *New England Journal of Medicine,* 352: (4), 380-391.

[21]. **•Karraouan, B., Bouchrif, B., Ziyate, N., Talmi, A., Yahia, K. I. S., Cohen, N., *et al.* (2009).** Evaluation of multi-plate microbial assay for the screening of antibacterial residues in poultry muscle. *European Journal of Scientific Research,* 35: (2), 311-317.

[22]. •Khaskheli, M., Malik, R. S., Arain, M. A.,

a. Soomro, A. H. & Arain, H. H. (2008).

[23]. Detection of β - Lactam Antibiotic Residues in Market Milk. *Pakistan Journal of Nutrition,* 7: (5), 682-685.

[24]. **•Kress, C., Seidler, C., Kerp, B., Schneider, E. & Usleber, E. (2007).** Experiences with an identification and quantification program for inhibitor-positive milk samples. *Analytica Chimica Acta,* 586: (1-2), 275-279.

[25]. **•Kunda, B., Pandey, G. S., Mubita, C., Muma, J. B. & Mumba, C. (2015).** MC. Compositional and microbial quality of heat-treated milk brands marketed in Lusaka, Zambia. *Livestock Research for Rural Development*.

[26]. **•Kuo, T.-F., Wang, Y.-K., Sheu, S.-Y., Chang, M.-H. & Wang, J.-H. (2009).** Detection of residues by use of solid-phase extraction and reversed phase high-performance liquid chromatography after oral administration of amoxicillin in bass muscle. ***臺灣獸醫學雜誌,*** 35: (2), 66-74.

[27]. **•Kurjogi, M., Issa Mohammad, Y. H., Alghamdi, S., Abdelrahman, M., Satapute, P. & Jogaiah, S. (2019).** Detection and determination of stability of the antibiotic residues in cow's milk. *PLOS ONE,* 14: (10), e0223475.

[28]. **•Makarem, H., Leboudy, A. & E, M. (2020).** Antibiotics Residues of Some Famous Antibiotics in Raw Milk of Different Species Sold at Local Markets. *Alexandria Journal of Veterinary Sciences,* 64: (2), 72.

[29]. **•Mirlohi, M., Aalipour, F. & Jalali, M. (2013).** Prevalence of antibiotic residues in commercial milk and its variation by season and thermal processing methods. *International Journal of*

Environmental Health Engineering, 2: (1), 41.

[30]. •**Mottaghiyanpour, E., Aminzare, M., Banikhademi, S. & Hassanzad Azar, H. (2018).** Direct screening of antibiotic residues in pasteurized, sterilized and raw milk supplied in Zanjan market, Iran. *Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii,* 28: (1), 22-28.

[31]. •**Movassagh, M. H. & Karami, A. R. (2011).** Beta-lactam antibiotics residues in pasteurised milk by Beta Star test in the north west region of Iran. *ARPN J Agri Biol Sci,* 6: 7-10.

[32]. •**Novak, R., Charpentier, E., Braun, J. S. & Tuomanen, E. (2000).** Signal Transduction by a Death Signal Peptide. *Molecular Cell,* 5: (1), 49-57.

[33]. •**Oruç, E. (2005).** Meningoencephalitis Tuberculosa in a Holstein Cow. *Veterinary Pathology,* 42: (6), 856-858.

[34]. •**Patel, N. M., Kumar, R., Savalia, C. V., Desai, D. N. & Kalyani, I. H. (2020).** Dietary exposure and risk assessment of antibiotics residues in marketed bovine raw milk.

[35]. •**Phillips, I., Casewell, M., Cox, T., De Groot, B., Friis, C., Jones, R., *et al.* (2004).** Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *Journal of Antimicrobial Chemotherapy,* 53: (1), 28-52.

[36]. •Prescott, J., Baggot, J. & Walker, R. (2000).

[37]. Antimicrobial Therapy in Veterinary Medicine (3rd ed). *Iowa: Iowa State University Press. Ames,,* 277.

[38]. •**Priyanka, V. J. J., Chauhan, S. L. & Garg, S. R. (2019).** Analysis of penicillin residues in milk using high performance liquid chromatography.

[39]. •**Quehl, A. (1977).** L. M. Lampert: Modern Dairy Products — Composition. Food Value. Processing Chemistry. Bacteriology. Testing. Imitation Dairy Products. I. Auflage, 446 Seiten, 84 Abb., 64 Tab. Chemical Publishing Company, Inc., New York 1975, Preis: 13, 50 £. *Food / Nahrung,* 21: (10), 967-967.

[40]. •**Rama, A. (2011).** Three years of surveillance (2008-10) for the assessment of contaminants residues in milk produced in Kosovo.

[41]. •**Ramírez, A., Gutiérrez, R., González, C., Escobar, I., Castro, G., Díaz, G., *et al.* (2001).** Detección de antibióticos en leche comercializada en la ciudad de México. *Rev. Salud Anim,* 23: (1), 37-41.

[42]. •**Rinken, T. & Riik, H. (2006).** Determination of antibiotic residues and their interaction in milk with lactate biosensor. *Journal of Biochemical and Biophysical Methods,* 66: (1-3), 13-21.

[43]. •**Ruegg, P. L. (2009).** Management of mastitis on organic and conventional dairy farms1. *Journal of Animal Science*, 87: (suppl_13), 43-55.

[44]. •**Sulejmani, Z. & Shehi, A. (2012).** Abuse of Pharmaceutical Drugs-antibiotics in Dairy Cattle in Kosovo and Detection of their Residues in Milk. *Journal of Ecosystem & Ecography*, 02: (04).

[45]. •**Tajick, M. A. & Shohreh, B. (2006).** Detection of Antibiotics Residue in Chicken Meat Using TLC. *International Journal of Poultry Science,* 5: (7), 611-612.

[46]. •**Tempini, P. N., Aly, S. S., Karle, B. M. & Pereira, R. V. (2018).** Multidrug residues and antimicrobial resistance patterns in waste milk from dairy farms in Central California. *Journal of Dairy Science,* 101: (9), 8110-8122.

[47]. •**Tolentino, R. G., Pérez, M. N., González, G. D., Vega, S., Lopez, M. G. & Flores, G. P. (2005).** Determination of the presence of 10 antimicrobial residues in Mexican pasteurized milk. *Interciencia,* 30: (5), 291-294.

[48]. •**Yıldız, Ö. (2008).** Investigation of the pyhsical and chemical properties of milk containing antibiotics.