Eco. Env. & Cons. 26 (4) : 2020; pp. (1515-1519)
Copyright@ EM International
ISSN 0971–765X

Using elisa to detect beta-lactam residue in wastewater discharge in the dairy factories of Mosul

Mazin N. Fadhel* and Ayman Albanna

College of Environmental Science and Technology, University of Mosul, Mosul City, Iraq

(Received 24 July, 2020; accepted 7 September, 2020)

ABSTRACT

The presence of veterinary antibiotic traces in the aqua environment leads to strong belief that negative effects are imminent against human beings. Dairy products factories in Mosul city, Iraq, often use contaminated milk which leaves some trace of antibiotic in the wastewater discharge. So, the purpose of this study was to estimate the concentration of the beta-lactam in the wastewater discharge based on before and after treatment. Sixteen dairy factories were chosen randomly from different districts of town. The specimens were examined according to sandwich ELISA method. The study lasted for 9 months (winter, spring and summer), to focus on the season effect. Results indicated that there were relative variations in the concentration of beta lactam, according to the different seasons, where the concentration of this element ranged from 0.00 -39.50 ng/mL of the drained water. It was noticed that the concentration of beta lactam increases during winter and summer seasons, accompanied with a significant decline during the spring season. However, the proportion of samplesare free of beta lactam were only 10.41%. On the other hand, the samples of water contained about 10 ng/mL or less formed only 54.16%, also, the high concentration of beta lactam higher than 10 ng/mL was 39.58% of all samples under study. The water treatment post in any of those factories, actually, were not so efficient in grasping the harmful elements such as the beta-lactam, where the filtration rates were not more than 0.02% (considered so nil), in this sort of matter.

Key words : *Public health, Beta lactam, ELISA, Dairy factory.*

Introduction

Although antibiotics are so functional in treating many bacterial diseases, for long period of time in both infected animals and human, they could cause some harm to the environment, which in reality is considered as environmental contaminants, especially in the developing countries (Albanna *et al.,* 2020; Al-Qatan*et al.,* 2019; Baquero*et al.,* 2008; Banoon *et al.,* 2020). The use of animal products by their owners, especially during the treatment period, leads to elimination of antibiotics through the digestive and urinary systems, in addition milk shedding through udder (Tempini *et al.,* 2018). At the same time, the owners are not so are not disciplined in following the use of antibiotics during withdrawal period of the drug. This often leads to contamination of the animal products with it, and effects indirectly to the environment during using by dairy factory without examining (Schwarz *et al.,* 2001). The residue of antibiotics in the drained water or the commercial dairy factories, often added to the milk by the plants owners, in order to lengthen the age of milk (Smith-Howard, 2016). It is important to realize that the powder milk used in many dairy products plants, which may contains a high level of antibiotics, resulting in obvious harm to the human beings and to the environment (Kneebone *et al.,* 2010). A Continuous accumulation of the beta lactam in human body and farm animals, despite

the low concentration may cause various diseases, such as cancers and babies deformities (Mÿburgh and Bütow, 2009; Smith *et al.,* 2002). Also, it was pointed out by Poole, (2004) that, if it happened not to get rid of all these harmful residues, the bacteria will gain multi resistance against different kinds of antibiotic, such as penicillin and cephalosporin. On the other hand, the ability of beta lactam structures to deter the bacterial growth and multiplication is kind of ineffective due to the presence of bacterial enzymes action like Beta-lactamase, as results of continued using same antibiotic or random treatment (Medeiros, 1997). Studies in this respect, revealed that the beta lactam residues, are present in different sources of water, including surface water, underground water and drainage water (Fatta-Kassinos *et al.,* 2011; Kemper, 2008). Technically, there are several laboratory techniques to identify the antibiotics from different samples including HPLC, LC, four-plate test, chemical reagents, and serological methods (Hark-Khan and Moats, 1995; Alkan, 2008; Okerman *et al.,* 2007; Zhang *et al.,* 2017). The modern dairy product plants should be equipped with highly sophisticated filtration instrument, ensure the trapping of antibiotic and other harmful elements (Xu *et al.,* 2007). This study aimed to estimate the antibiotics, quantitatively, in the drainage water of some dairy products factories in Mosul city, Iraq, for three successive seasons (winter, spring and summer).

Materials and Methods

Sixteen dairy products factories were chosen from different locations in Mosul city, Iraq, to evaluate the water discharge of its possible content of antibiotics from dairy products process. Forty-eight samples were secured during 3 different seasons, winter, spring and summer, where 5 mL of discharged water before having it pumped into the filtrating device, as an input, and 5 mL of the same water after being out of the filter device, as an output, were secured to do the analytical study. The samples were immediately transferred in special freezers (-20 °C), till were subjected to the ELISA analysis to determine the level of antibiotics as (Abuknesha and Luk, 2005). From the ELISA lab technique, it was able to measure the residues of beta lactam in the input and output water discharged, both quantitatively and qualitatively. The principal of enzyme linkage immune sorbent assay based on the interaction between the antigen (Ag) and antibody (Ab) reaction.

To do the detection of beta lactam, based on the enlisted company instructions, the samples were prepared in wells plate at 20-25 °C, along with the preparation of control wells. Taking into consideration, that all substances required to be under the same conditions.

Also, an addition of standard control parameter was taken place, where each sample was tested by adding 50 microliter which consisted of (10 microliter water sample and 40 microliter Standard Solution). Then after, 100 µL of HRP-conjugate reagent added to each well and incubated at 37 °C for one-hour period, with repeated washing of all wells four times, using wash Solution (400 µL).

After that, the plate inverted and dried against towel papers. An amount of 50 µL of Chromogen solution and 50 µL Chromogen solution B was added to each well. Gently, the plate was shacked and incubated at 37 °C for 15 min. Finally, 50 µL of Stop Solution was added to all plate wells, where all the optical density data were read very at 450 nm directly by using plate reader.

Resultant data were subjected to T test statistical analysis for determination the differences in proportions of beta lactam values among seasons and pre and after being subjected to the filtration procedure, as well.

Results

Results indicated that the majority of the examined samples obtained from the various dairy products plants,under this study, contained some residues of beta lactam which are confirmed to be descended from the contaminated milk. Only 10.41 % of all samples were free of beta lactam. On the other hand, 54.16% of all the tested samples showed less than 10 ng/mL of the beta lactam,as illustrated in Table 1. The high level of beta lactam (more than 10 ng/mL) was significantly, $P < 0.05$, demonstrated in 39.58% of all the tested samples.

Regardless of season effect, it was found that the treatment stations within these plants were not so efficient in filtrating the high rate of beta lactam residues, where only 0.02% of the beta lactam was filtered out. Figure 1 shows a linear distribution of the beta lactam concentrations in the before and after processing water discharged in the dairy products, under this study. Regarding the season effect, it was

Table 1. The beta lactam values for dairy factories water discharged detected by using the ELISA technique.

Dairy factory ng/ml	Winter		Spring		Summer	
	Before processing	After processing	Before processing	After processing	Before processing	After processing
Sample 1	0.23	0.21	0.12	0.11	11.85	10.34
Sample2	1.49	1.23	0.97	0.87	18.09	17.87
Sample 3	30.01	27.3	2.53	2.01	3.76	3.55
Sample 4	2.98	2.65	2.03	2.02	0	0
Sample 5	0.92	0.85	1.76	1.55	9.04	8.76
Sample 6	22.76	20.27	2.55	2.27	17.86	16.99
Sample 7	12.63	11.03	0	0	0.32	0.11
Sample 8	37.47	33.82	1.67	1.11	36.7	33.03
Sample 9	0.11	0.09	0.78	0.54	0.65	0.573
Sample 10	0	0	1.11	0.98	19.72	19.03
Sample 11	27.21	23.54	18.76	15.66	39.5	37.63
Sample 12	0.29	0.23	1.45	1.39	4.27	3.89
Sample 13	0	0	4.65	4.06	0	0
Sample 14	0.06	0.05	6.79	6.3	33.87	32.45
Sample 15	0.37	0.24	0.56	0.487	11.87	10.94
Sample 16	29.07	28.35	13.45	12.78	0.25	0.19

noticed that there were similar levels of beta lactam declination, in the water discharged of the three successive seasons, as indicated in Table 2. Also, dissimilar trend was found in the comparing the values beta lactam concentration for before and after water discharged within each season. The differences were statistically significant at P<0.05. On the other hand, the least significant concentration of the beta lactam level in the water discharged, found in this study, was during the summer season as indicted in Figure 2.

Discussion

The concentrations of beta lactam were significantly higher during the winter and summer seasons in comparison to their counterparts during the spring season. The possible explanation for this phenomenon, probablydue the fact that lactating farm animals mostly get infected with *Staphylococcus* spp, *Salmonella* spp and others bacteria during the days of those seasons (Songer and Post, 2004). Also, mastitis and viral infections may have a role in rendering the farm animals owners tending to overuse of antibiotics, such penicillin, cephalosporin and others in treating their affected animals (Endimiani *et al.*, 2012; Du Preez, 2000). As it is well scientifically known, that these sorts of anti-bacteria drugs (broad spectrum antibiotics) have a vast capabilities of suppressing gram positive and gram negative bacteria (Prescott and Baggot, 1988). On the other hand, the trivial levels of beta lactam detected in the water discharged of the majority of the dairy products plants, under this study, indicating that there is a substantial lack in the efficiency of the side water filtration station within the plant (if actually present) to biologically dispose the antibiotics and/or disinfectants and getting them out in the water discharged (Ata and Töre, 2019). In conclusion, it is necessary to adopt real solutions to deter such risky

Table 2. Concentrations means of beta lactam ng/L, in water (pre and after processing water discharged) from 16 dairy products plants over three seasons, in Mosul city.

Seasons	Beforeprocessing	After processing	Difference
Winter	10.350 ± 3.456	9.366 ±3.153	0.984 ± 0.342*a
Spring	3.699 ± 1.307	3.259 ±1.147	0.440± 0.186 *a
Summer	12.984 ± 3.408	12.210 ± 3.212	0.775± 0.106 *a
Overall mean	9.011 ± 1.736	8.279 ± 1.60	0.733 ± 0.153 *

*Significant at <P 0.05 (within season)

1-Means+SE, among seasons, with similar small-case letter are statistically similar (P< 0.05)

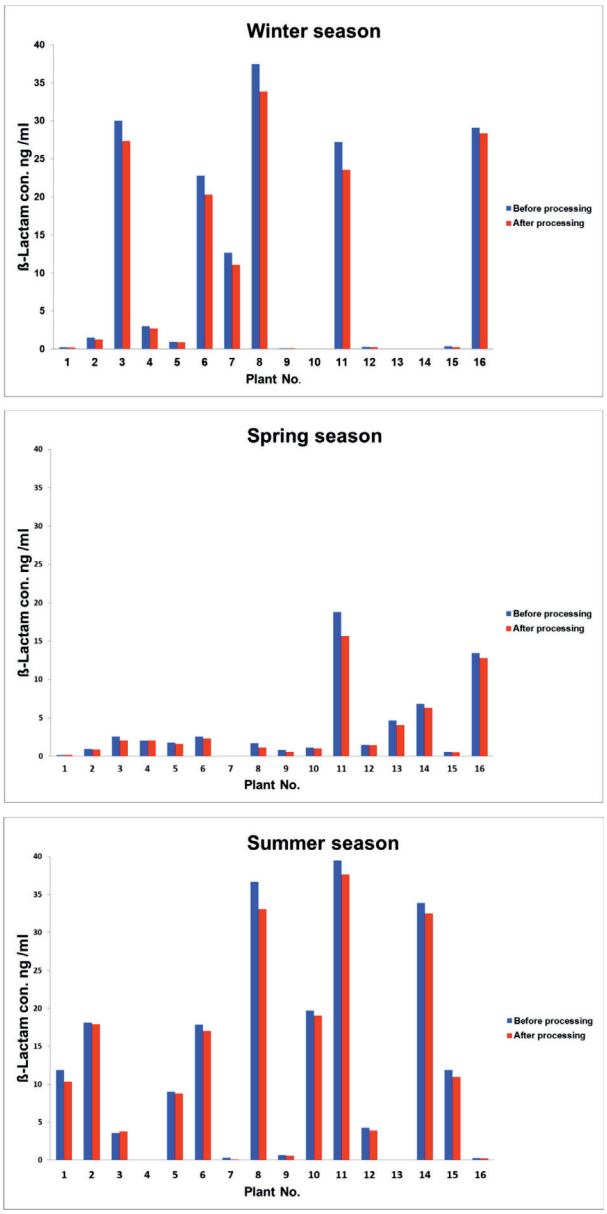

Fig. 1. The proportions of the quantities of beta-lactam before and after processing water discharged, according to different seasons

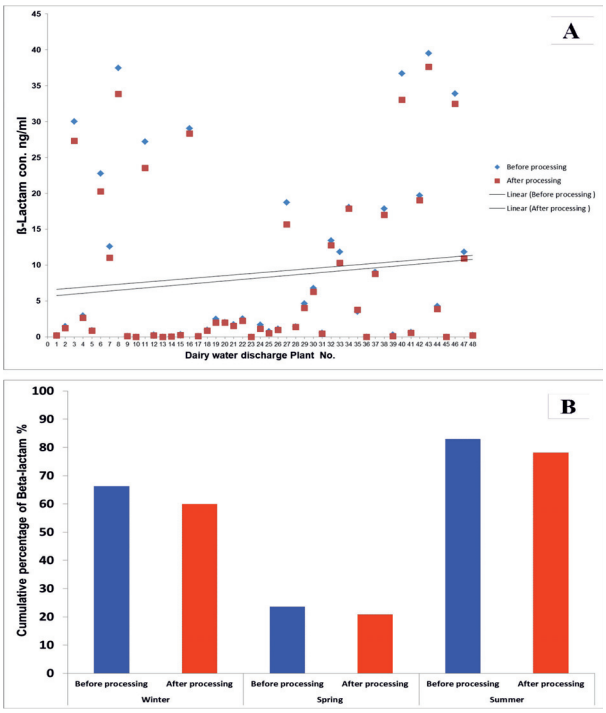

Fig. 2 A. Linear distribution between the before and after water treatment plant.
B: A cumulative percentage beta lactam concentrations among dairy factories, before and after treatment by the sub-stations inside the plants.

problems where polluted water is dumped into the environment. This can be easily done by installation of additional units in the dairy products plants to prevent the leakage of harmful elements, antibiotics in particular. Also, thinking of farm animals being the chief cause of the antibiotic problem is another matter needs to deal with. We suggest to pursuing more studies in this field of human health hygiene and environment safety, to confirm the results obtained in this investigation.

Conclusion

Since we have dealt with the samples based on pre and after treating the water in the factories subjected to this study. And we have found highly significant concentration of beta lactam, it is strongly advising pursue such research on large scale basis. Accordingly, it might be so necessary to take some measurement and precautions by the official to a void the passing of the harmful elements and transmission to the environment.

Acknowledgment

The authors are very grateful to the University of Mosul / College of Environmental science and Technologies for their provided facilities, which helped to improve the quality of this work.

References

Abuknesha, R.A. and Luk, C. 2005. Enzyme immunoas-

says for the analysis of streptomycin in milk, serum and water: development and assessment of a polyclonal antiserum and assay procedures using novel streptomycin derivatives. *Analyst*. 130(6) : 964-970.

Albanna, A., Fadhel, M.N. and Al-Shaker, Y.M. 2020. August. Detection Beta-lactam residues in imported and local dairy products in Mosul city. In: *IOP Conference Series: Earth and Environmental Science* (Vol. 553, No. 1, p. 012014). IOP Publishing.

Alkan, P. 2008. *The confirmation of the commercial kits used in the detection of antibiotics in milk with HPLC (high pressure liquid chromatography)* (Master's thesis, Izmir Institute of Technology).

Al-Qatan, M.A.R., Al-Khayyat, M.Z. and Saeed, H.K. 2019. Isolation of Antibiotic-Resistant Bacteria from Sewage Water in Mosul City. *Biochemical and Cellular Archives.* 19(1) : 1415-1418.

Ata, R. and Töre, G.Y. 2019. Characterization and removal of antibiotic residues by NFC-doped photocatalytic oxidation from domestic and industrial secondary treated wastewaters in Meric-Ergene Basin and reuse assessment for irrigation. *Journal of Environmental Management*. 233 : 673-680.

Banoon, S., Ali, Z. and Salih, T. 2020. Antibiotic resistance profile of local thermophilic *Bacillus licheniformis* isolated from Maysan province soil. *Comunicata Scientiae*. 11 : e3921-e3921.

Baquero, F., Martínez, J.L. and Cantón, R., 2008. Antibiotics and antibiotic resistance in water environments. *Current Opinion in Biotechnology*. 19(3) : 260-265.

Du Preez, J.H. 2000. Bovine mastitis therapy and why it fails: continuing education. *Journal of the South African Veterinary Association*. 71(3) : 201-208.

Endimiani, A., Bertschy, I. and Perreten, V. 2012. Escherichia coli Producing CMY-2 β-Lactamase in Bovine Mastitis Milk. *Journal of Food Protection*. 75(1).

Fatta-Kassinos, D., Meric, S. and Nikolaou, A. 2011. Pharmaceutical residues in environmental waters and wastewater: current state of knowledge and future research. *Analytical and Bioanalytical Chemistry*. 399(1) : 251-275.

Hark-Khan, R. and Moats, W.A. 1995. Identification and measurement of â-lactam antibiotic residues in milk: integration of screening kits with liquid chromatography. *Journal of AOAC International*. 78(4) : 978-986.

Kemper, N. 2008. Veterinary antibiotics in the aquatic and terrestrial environment. *Ecological Indicators*. 8(1): pp.1-13.

Kneebone, J., Tsang, P.C. and Townson, D.H. 2010. Rapid antibiotic screening tests detect antibiotic residues in powdered milk products. *Journal of Dairy Science*. 93(9) : 3961-3964.

Medeiros, A.A. 1997. Evolution and dissemination of β-lactamases accelerated by generations of β-lactam antibiotics. *Clinical Infectious Diseases*. *24*(Supplement_1) : S19-S45.

Mÿburgh, H.P. and Bütow, K.W. 2009. Cleft soft palate reconstruction: prospective study on infection and antibiotics. *International Journal of Oral and Maxillofacial Surgery*. 38(9) : 928-932.

Okerman, L., Van Hende, J. and De Zutter, L. 2007. Stability of frozen stock solutions of beta-lactam antibiotics, cephalosporins, tetracyclines and quinolones used in antibiotic residue screening and antibiotic susceptibility testing. *Analytica Chimica Acta*. 586(1-2) : 284-288.

Poole, K. 2004. Resistance to â-lactam antibiotics. *Cellular and Molecular Life Sciences CMLS*. 61(17) : 2200-2223.

Prescott, J.F. and Baggot, J.D., 1988. *Antimicrobial Therapy in Veterinary Medicine*. Blackwell scientific publications.

Schwarz, S., Kehrenberg, C. and Walsh, T.R. 2001. Use of antimicrobial agents in veterinary medicine and food animal production. *International Journal of Antimicrobial Agents*. 17(6) : 431-437.

Smith-Howard, K. 2016. *Pure and Modern Milk: An Environmental History Since 1900*. Oxford University Press.

Smith, D.M., Kazi, A., Smith, L., Long, T.E., Heldreth, B., Turos, E. and Dou, Q.P. 2002. A novel β-lactam antibiotic activates tumor cell apoptotic program by inducing DNA damage. *Molecular Pharmacology*. 61(6) : 1348-1358.

Songer, J.G. and Post, K.W. 2004. *Veterinary Microbiology-E-book: bacterial and fungal agents of animal disease*. Elsevier Health Sciences.

Tempini, P.N., Aly, S.S., Karle, B.M. and 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.

Xu, W., Zhang, G., Li, X., Zou, S., Li, P., Hu, Z. and Li, J. 2007. Occurrence and elimination of antibiotics at four sewage treatment plants in the Pearl River Delta (PRD), South China. *Water Research*. 41(19) : 4526-4534.

Zhang, K., Zhou, X., Du, P., Zhang, T., Cai, M., Sun, P. and Huang, C.H. 2017. Oxidation of β-lactam antibiotics by peracetic acid: Reaction kinetics, product and pathway evaluation. *Water Research*. 123 : 153-161.