WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 4, 1494-1500. Research Article ISSN 2277– 7105

# DETECTION OF GENTAMYCIN RESIDUE LEVELS IN MASTIC COW'S MILK FOLLOWING INTRAMAMMARY ADMINISTRATIONS

**Samia A. A. Hassan[1], Sania A. I. Shaddad[2]* and Huda O. Ali[3]**

[1]Central Veterinary Research Laboratory, Department of Biochemistry Nutrition Toxicology and Pharmacology, P.O. Box 8067, Alamarat, Khartoum.

[2]Prof. of Pharmacology, Faculty of Medicine, Dep. of Pharmacology, University of Khartoum, P.O. Box 102, Sudan.

[3]Central Veterinary Research Laboratory, Department of Bacteriology, P.O. Box 8067, Alamarat, Khartoum.

Article Received on
14 Feb. 2021,

Revised on 06 March 2021,
Accepted on 26 March 2021

DOI: 10.20959/wjpr20214-19698

***Corresponding Author**
**Prof. Sania I. A. Shaddad**
Prof. of Pharmacology, Faculty of Medicine, Dep. of Pharmacology, University of Khartoum, P.O. Box 102, Sudan.

## ABSTRACT

The excessive use of antimicrobial drugs in dairy farm may result in the presence of their residues in milk and milk products, which can adversely affect public health. This study was conducted to determine the presence of gentamycin residues in cow Milk samples after treated with intramammary infusion for mastitis. Information about treatment and drugs were estimated by questionnaires and direct interview with farm's owner. A total of 300 milk samples was collected during the period from September 2018 to October 2019 of 200 farms in Khartoum north (100 samples), Khartoum (100) and Elgazeera state (100) samples for 5 days after the last administration. The milk samples were screened for gentamycin residues using Delvo®-test and a modifi one plate test (O.P.T.) using *Bacillus subtilis* (BGA-DSM-618) as a test organism. The result indicated that out of 300 milk samples were positive for antibiotic residues (83%) of these were in Egazeera (69%) were in Khartoum north and lowest percentage were in Khartoum (39%). To obtain the gentamycin concentrations the inhibition zones diameter were interpreted with gentamycin standard curve. Gentamycin levels were evaluated in different times. Highest levels were noted at first day while the lowest were in fifth day after withdrawal period which were greater than its international maximum residues limit (MRL).

## INTRODUCTION

Aminoglycoside are a group of synthetic antimicrobial agents with broad spectrum activity against a wide range of most gram-Negative and positive bacteria (Goljan Edward, 2001) and used in human and Veterinary Medicine for therapeutic and prophylactic purpose.

Anti-microbial drugs have played an important role in the field of animal husbandry and agro-industry, therefore, the benefit of improved productivity in food of animal origin is not obtained without reduced the risk associated with veterinary drug residues that remain in treated animals (Craw, 1985) and may poses a health hazard to the customer (Chanda, 2014) such as allergic reaction, disturbance of intestinal microflora, bacterial resistance, Neurological and toxicological disorders (Kirbis, 2006), (Babapour. 2012) and (Vande, 2000). The presence of antibiotic residues in food stuff above the maximum residues limits (MRLs) was recognized by national and international public health agencies to ensure Human health, process product quality, safety and free from other contaminants (Boor, Murphy, 2000), (Salih, 2006). Gentamycin is produced commercially by the fermentation of *Micromonospa purpurca* (WHO, 1995) Gentamycin sulphate was active against wide range of bacterial infections (Gomes & Honriques, 2015), and used to treat cystitis, urethritis, endometritis and mastitis in livestock it was absorbed quickly and retained in blood and tissue for 12-24$^{th}$ hours and eliminated unchanged with urine (Archimbault, 1983). Intramammary infusion of gentamycin sulfate is the most common therapy to control or prevent mastitis disease in dairy farms (Wilson *et al*., 1998) and was cited as a major reason of milk contaminations (McEwen *et al*., 1991). Gentamycin residues in milk may alter the processing qualities of raw milk by inhibiting starter cultures use in milk preparation of cheese and other fermented dairy products (Brady and Katz, 1988). World Health Organization (WHO) and food and drug administration (FDA) have set maximum residue limits for human consumption as (200µg/L) for gentamycin sulfate. The aim of this study was to evaluate the presence of gentamycin residues in bovine milk sample after therapeutic treatment with intramammary infusion.

## MATERIAL AND METHODS

### Study area

This study was carried out in dairy farms in Khartoum State (Khartoum and Khartoum north) and ElGezieera State which lies longitudes 32$^{o}$ 22$^{o}$-43 20'$E_2$, South Khartoum State.

**Samples collections**

The knowledge about the disease and treatments were estimated by questionnaire and direct interview with Farm's Owner. A total of 300 milk samples were collected from 200 dairy farms during October 2016 to November 2017. Milk samples were collected from each mammary quarters of mastitic cow treated with gentamicin sulfate (170mg equal to gentamycin 100mg) as intramammary infusion for five consecutive days. The samples were taken at 1h, 12h., 24h, 1$^{st}$ day, 2$^{nd}$, 3$^{rd}$, 4$^{th}$ and 5$^{th}$ day after the last administration of the drug in sterile plastic containers and placed in a thermo-flast-containers, frozen at -20C$^{o}$ until analysis at CVRL labs.

**Detection of gentamycin residues in milk samples**

The commercial microbiological inhibitor test was used (Delvo® -test SPNT, Netherlands). To detect antimicrobial agents (Peter *et al*., 2003).

The test ampoule containing nutrient agar seeded with *Bacillus stearothermophilus* spores and Bromocresol purple as indicator, was used. 100 µg of test sample was added and incubated for 3 hours. A purple color indicated the presence of gentamycin residue (positive results) at or above the detection limits of the test while the yellow color indicates that the antibiotics which are below the detection limits (Negative result). The change of color purple to yellow (False-positive results) which was confirmed by heating the samples to 82C$^{o}$ in water bath for 10 minutes then 100µg of samples were added to test ampoule of the Delvo®-test sp. (Molina *et al*., 2003).

**Microbiological assay methods**

The modified one-plate test described by Koenen-Dierick *et al*., 1995 was used to determine the concentrations levels of gentamycin sulfate in milk samples.

The blank samples were spiked with different serial of know concentrations of standard gentamycin sulfate for calibration curve. Milk samples were screened microbiologically using *Bacillus* subtilis BGA-DSM-618 as a test organism. 0.1micoliter (µg/L of sample was placed into Tuplicate wells on sterilized agar plate medium and incubated at 37$^{o}$C for 18-24hrs. The mean diameter of inhibition zones around the test sample were measured in (MM), and plotted versus calibration standard curve to evaluate the gentamycin levels in milk.

**Statistical analysis**

The statistical analysis of the data obtained was carried out using ANOVA with significance difference P<0.05). Chi-square was used to treat the results obtained by Delvo®test.

**RESULTS**

Microbiological tests showed that 191(63.7%) and 170(56.7%) for Delvo®-test and one-plate test out of 300 samples were positive for gentamycin residue respectively.

The higher positive percentages were recorded in ElGazeera State 83% and Khartoum North 69% while Khartoum showed lower positive percentages 39% in all sample screened by Delov®-test compared with that results 77%, 58% and 35% in ElGazeera state, Khartoum North and Khartoum respectively obtained by one –plate tests (table 1) Fig .(1).

**Table 1: The percentage of positive milk samples examined for gentamycin residues ElGazeera State, Khartoum-north and Khartoum State.**

| | No. of samples | Delvo®-test (%) | One-plate test 100% |
|---|---|---|---|
| ElGazeera State | 100 | 83(83%) | 77(77%) |
| Khartoum-North | 100 | 69(69%) | 58(58%) |
| Khartoum State | 100 | 39(39%) | 35(35%) |
| Total | 300 | 191(63.7%) | 170(56.7%) |

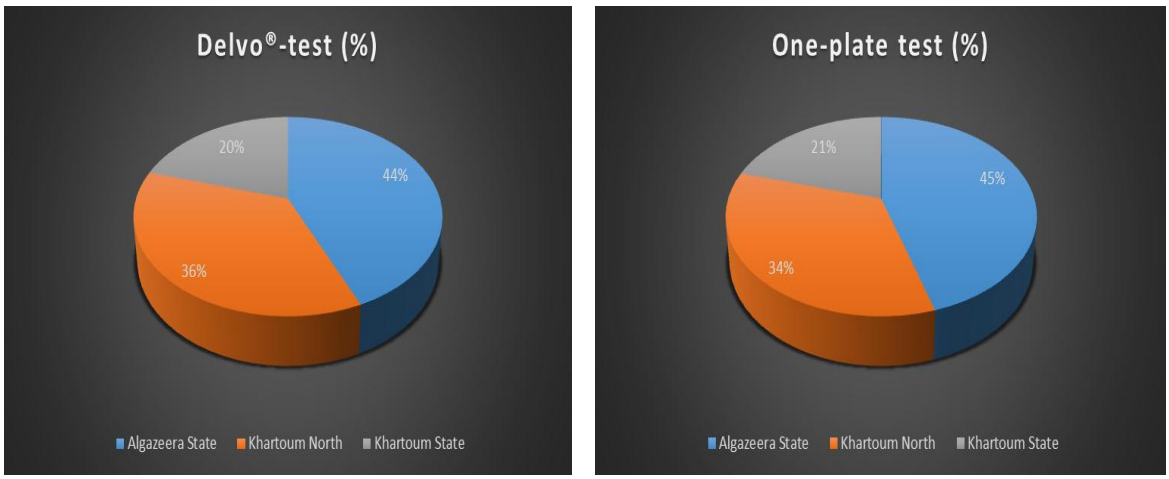


**Fig. 1: The percentage of positive milk samples examined for gentamycin residues ElGazeera State, Khartoum-north and Khartoum State.**

**Table 2: Mean ±SEM of gentamycin concentrations in milk samples collected for treatment of mastitis with intramammary infusion.**

| Study area / Time | 12h | 24h | 1st | 2nd | 3th | 4th | 5th |
|---|---|---|---|---|---|---|---|
| Algazeera | 3.86±12.73 | 2.98±6.20 | 2.13±12.81 | 1.59±.15 | 1.05±.15 | .89±.13 | 0.09±.22 |
| Kh. North | 3.60±.122 | 2.35±12.2 | 1.98±13.1 | 1.18±.31 | 1.01±1.8 | 0.4±0.15 | 0.07±32 |
| Khartoum | 3.51±13.1 | 2.27±8.2 | 1.20±.15 | 0.96±12.00 | 0.64±.1 | 0.027±.6 | 0.06±0.07 |

The comparison of inhibition Zones diameters in (mm) of positive samples with the calibration curve of standard gentamycin concentrations were summarized in Table (2).

The significant higher concentrations were observed after 12h of last administration were 3.86±12.73, 3.60±.122 and 3.51±13.1mg/L mol in ElGazeera, Khartoum north and Khartoum respectively while the lowest concentration of gentamycin residues observed in day 5 after treatment were 0.09±.22, 0.07±.23 and 0.06±0.01 in AlGazeera State, Khartoum-north and Khartoum respectively. The concentrations levels persist a longer period after withdrawal time than that has been established for milk by WHO.

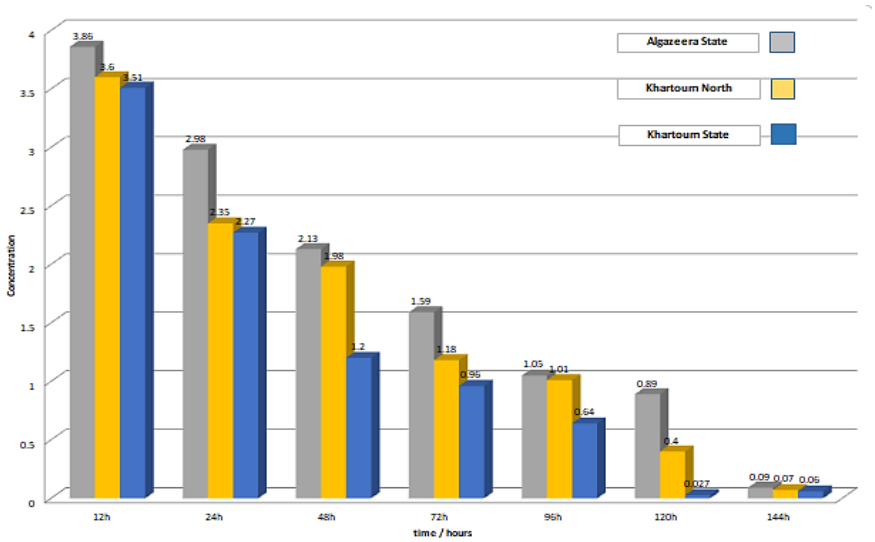


**Fig. 2: Mean ±SEM of gentamycin concentrations in milk samples collected for treatment of mastitis with intramammary infusion.**

## DISCUSSION

The excessive use of intramammary infusions for treatment or prevention of mastitis which is the most prevalent disease in lactating cattle and this may be the reason of milk contamination. The disease status of an animal and the route in which drugs are administered influence the potential for residues. (Kaneene and Miller, 1997) disease condition may affect

the pharmacokinetics of the drug, its metabolism and elimination, which may cause the drug to accumulate in affected tissues (Kaneene and Miller, 1997).

The highest percentage of the positive samples detected for gentamycin residues in ElGazeera (83%) and Khartoum north (69%) were similar to the other previous studies mentioned by (Elzabeir, 2011), (AbdelRaman, 2001) and Adil, M. 2012) whom they found that the 84.7% and 76.6% of samples collected from treated cow with intramammary infusion were positive for antibiotic residues and this may be attributed to large doses of antibiotic and more frequent of treatment by farm owner. These in contrast with the results obtained by (Tyckowska *et al*., 1989), this may be due to the difference in type of drug formulation, dosage and method of detection. Our result was closely similar to that reported by (Said Ahmed, 2008) who found that 39% of the examined samples for residues in Khartoum, this Finding were different from that of Edima *et al*., 2012) who reported that 32% of samples contaminated with gentamycin residues in Nyala the difference may be explained the awareness and good hygiene status of the farms.

Furthermore, the significant higher occurrence of gentamycin concentrations above the maximum residue limited (MRL) (200µg/L) in screened milk samples may explained misuse of antibiotic and lack information about withdrawal period. In conclusion effective surveillance and monitoring of veterinary drug residues should be recommended. Inability control laboratory should be constructed for food of animal organ.

**REFERENCES**

1. Brady, M.S. Katz S.E. Antibiotic antimicrobial residue in milk. J. Food Prot, 1988; 51(1): 8-11.
2. Wilson, D.J., Sears, PM, Hutchinson, L.J. Dairy producer attitudes and farm practices used to reduce the likelihood of antibiotic residues in Milk and dairy beef: a five state survey. Vet. Clin. North AM large Amin. Pract, 1998; 19(5): 24-30.
3. Archimbault, P. (1983). Persistence in milk of active antimicrobial intrammary substances (in Ruckebushi, 1993.
4. WHO Evaluation of certain Veterinary drug residues in food: technical report 851. Geneva: WHO, 1995.
5. Gomes F.L. Hnriques M. Control of bovine mastitis: old and recent therapeutic approaches. Curr. Microbiol, 2015; 72(4): 377-382.

6. Kaneene, J.B. and R. Miller, Problems associated with drug residues in beef from feeds and therapy. Revue Scientifique et technique et office Internationale du Epizooti, 1997; 16: 694-708.
7. Mohammed, H. A. Effect of Husbandry practice on milk production in dairy farms in sharg Elneel locality. Khartoum State, M.Sc. thesis. University of Khartoum, 2011.
8. ElZubeir, Ibtisam, E.M. and Ahemd, Mahala, G. An over view of the management practices and constrains: at the dairy camps in Khartoum State, Sudan. Sudan Rovas, 2011; 1: 425.
9. Said Ahmed, A.M., Ibtisam, E.M. ElZubeir, O.A.O. Elowni and M.K.A. Ahmed Assessment of microbial loads and Antibiotic Residues in Milk supply in Khartoum State, Sudan Res. J. Dairy Sci, 2008; 2: 36.
10. Abdel Rahman, M.A. Detection of antibiotic in milk and effect of heating on the antibacterial activity. MSC thesis. Fac. of Vet. Med. U. of Sudan, 2001.
11. Tyczkowska, K., VoyKsner R.D. AronsonAL. Development of analytical method for penicillin G in bovine milk by liquid chromatography with ultraviolet visible detection and confirmation by mass spectrometric detection J. Chromatogr, 1989; 490(1): 101-113.
12. Edima, H., Tofaing, J.T., Ngoune, L.T. and Ndjouenkev, R. Assessment of antibiotic residue in cow milk produced in Ngoovundere. Inter. J. of Biolog., Pharmacy and Applied Sciences, 2012; 1(11): 1530-1538.
13. Goljan, Edward, Rapid Review pathology Philadelphia, PA: Elsevier. P, 2011; 242.
14. Marwa Ragab, 2015, Mai A.M., A.A., Shamima, H., Nahla Salah, Heba, M. Hassan, (2015). Validation of ARP-Hple method for estimation of gentamycin sulfate in poultry meat using UV detector. Egypt. J. Chem. Environ. Health, 2011; 1(1): 955-970.
15. Chanda, R., Fincham, R., Venter, P. Review of the regulation of veterinary drug and residues in South Africa. Crit, Rev. Food Sci., Nutr, 2014; 54: 488-494.
16. Babapour A., Azami. L. Fartashmehrj. Over view of antibiotic residues in beef and Mutton in Ardebi, North West of Iran. World Appl. Sci., 2012; 19: 1417-1422.
17. Salih H.I.M. Residue of antibiotic in poultry meat MSc. Thesis Dep. of Preventive Medicine and Veterinary Public Healthy Faculty UN of Khartoum, 2006.