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Assessment of Oxytetracycline and Penicillin G Residues Levels in Raw and Fermented Milk in Maiduguri, Northeastern Nigeria

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Abstract

This study was conducted to evaluate the presence and concentration of Oxytetracycline and Penicillin G residues in raw cow and Fermented milk consumed in Maiduguri, Northeastern Nigeria. A total of 172 (86 raw and 86 fermented) milk samples were randomly collected from different milk selling points in Maiduguri. A Spectrophotometer ultraviolet visible spectrum machine was used to detect and quantify the oxytetracycline and penicillin G residues in the milk samples. All samples (raw and fermented milk) tested positive for oxytetracycline and penicillin G residues with a mean residual concentration of 36 µg/L and 20 µg/L in raw and fermented milk respectively for oxytetracycline while the mean residue concentration of penicillin G is 649 µg/L and 397 µg/L in raw and fermented milk respectively. However, the oxytetracycline residues detected were all below the Maximum Residue Level (MRL) standards of 100 µg/L, while the penicillin G residue detected were all above the maximum residue standard of 5 µg/L. The maximum and minimum residue for oxytetracycline detected in raw milk was 79 and 10 (µg/L) respectively while the maximum and minimum residue detected in fermented milk was 42 and 9 (µg/L) respectively. The maximum and minimum residue detected for penicillin G residue in raw milk was 1993 and 767 (µg/L) respectively while the maximum and minimum residue detected in fermented milk was 288 and 164 (µg/L). This study revealed the presence of both oxytetracycline and penicillin G residues in raw and fermented milk consumed in Maiduguri. However, oxytetracycline residues detected in this study were found to be within the acceptable limits, while the penicillin G residues were highly above the MRL standard set by CODEX.

Keywords: Oxytetracycline, Penicillin G, Milk, Residue, Spectrophotometer, Maiduguri

INTRODUCTION

Globally, concerns about healthy and qualitative life are of supreme priority and one of the very imperative requirements is eating healthy, safe and nutritious diet (Wahl *et al*., 2017). Cow milk is known to make up a balanced and nutritious human diet based on its numerous nutrient compositions (Marangoni *et al.*, 2018). Cattle rearing, milking and its processing in Nigeria is mostly done by the nomadic and semi-nomadic Fulani herdsmen who without doubt engage in wrong and excessive use of veterinary drugs for preventive and treatment purposes in their cattle (Olatoye *et al*., 2016). In veterinary medicine, antibiotics are used at therapeutic levels largely to treat diseases and at sub therapeutic levels for prophylactic measures, to enhance feed efficiency and utilization and to promote growth (Khaskheli *et al*., 2008). Antibiotics such as penicillin G, amoxicillin and tetracycline have been adjudged to be the most frequently used antimicrobials for the prevention and therapy of mastitis in dairy cows, and thus are the most commonly found chemical residues (parent compound or its metabolites) in milk (Darwish *et al*., 2013). Despite their usefulness and efficacy, these chemotherapeutic agents can occur as residues in the treated animals if consumed before the elapsing of the withdrawal period (Priyanka *et al*., 2017).

While quite a lot of factors have contributed to the problems of antibiotic residues in food animals;

such as poor treatment record or a miss up that leads to inability to identify treated animals, the most infringements results from the use of these chemical agents in an irrational manner that is very different from the manufactures instruction. These occur primarily through not observing label withdrawal periods (Ondieki *et al.,* 2017) as well as extra label use of the drugs (Jayalakshmi *et al.,* 2017).

Residual deposits of these chemicals, therefore, may be much and this can lead to severe dangers to human health when consumed. Health effects such as reproductive disorders, problems of the bone marrow, allergic reactions, alteration of the normal intestinal flora, cancer, mutagenecity, nephropathy, hepatotoxicity, and antibiotic resistance might ensure (Nisha, 2008).

Evolvement of multidrug resistant super bugs due to misuse of antibiotics have led to the increase incidences of morbidity, futile or failed therapeutic efficacy, increase cost of health care (Brown *et al.*, 2020) making the scientific world occupied in the search for substitute to relieve or curtail this global threat.

In an effort to assure the safety of food meant for human consumption, most countries have established official standard laws aimed at strictly regulating the maximum residue levels (MRLs) of Veterinary drugs in animal products (Rana *et al*., 2019). These maximum residue limits are expected to standardize the maximum allowable levels of the drug residue for each antibiotic which is considered safe and tolerable in food animals (CAC, 2018). The Codex Alimentarius Commission have established safe levels of antimicrobials in edible tissues which is 100 (microgram/litre) µg/L for oxytetracycline in milk and 5 (microgram/litre) µg/L for penicillin G residues in milk (CCRVDF, 2013).

Considering that antibiotic residues in food can cause detrimental effects on consumers, quality control of all food products for these residues is highly imperative especially in developing countries like Nigeria. These are even more important in highly and widely consumed food like milk. Therefore, the aim of this study is to determine the presence and concentration of Oxytetracycline and Penicillin G residues in raw and fermented milk in Maiduguri, Northeastern Nigeria.

MATERIALS AND METHOD

STUDY AREA

This study was conducted in Maiduguri, a city in North eastern Nigeria. Maiduguri is located on latitude 11° 50´ North and longitude 13° 09´ East. Also called Yerwa by indigenous tribes, the city lies on somewhat flat topography part of the vast undulating plain which slopes gently towards Lake Chad (Hiribarren, 2017). It is the major trading centre of North eastern Nigeria. Its economy is largely based on services and trade with large scale livestock production. It is estimated to have a population of 1,065,000 as of 2016 (Britannica, 2019) and is occupied by people of diverse ethnic origins with indigenous Kanuri as the majority, Figure 1.

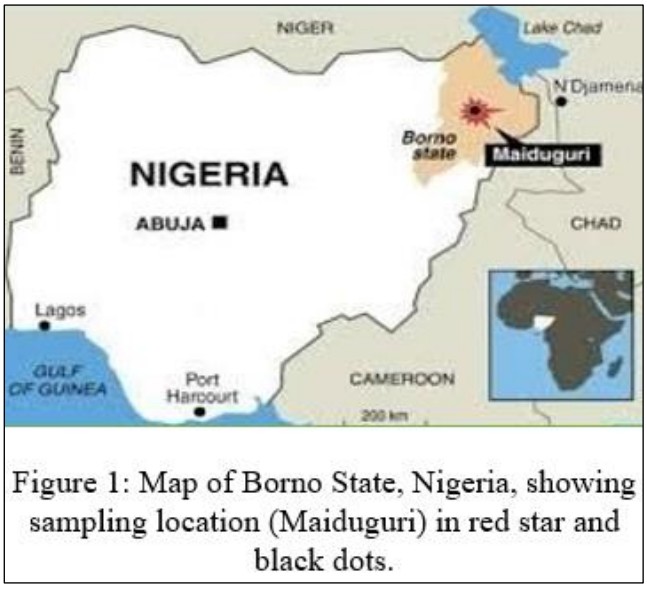

Figure 1: Map of Borno State, Nigeria, showing sampling location (Maiduguri) in red star and black dots.

SAMPLE SIZE DETERMINATION

The formula for random sampling by (Thrusfield, 2007) was used to determine the sample size with 99% confidence interval and 1% absolute precision as thus:

$$N = Z^2pq/d^2$$

Where,

N = Sample size

Z = Confidence interval

p = Prevalence= 42.6% (Olatoye *et al.*, 2016)

q = 1 – p
d = Absolute precision

$$N = \frac{2.582 \times 0.426 \times (1 - 0.426)}{0.01} = 163$$

Hence, a total of 163 sample size was determined and was rounded up to 172 for even distribution.

SAMPLE COLLECTION

Samples were collected between July and September. A total of 172 milk samples 86 each for fermented and raw milk were randomly collected from different milk selling points and mini cattle ranches from within Maiduguri. Fermented milk samples were obtained from Monday Market, Baga road Market, Bulumkutu "yan nono" Market, Molai market and Tashan Bama Market. Similarly, raw cow milk samples were obtained from small cattle ranch in Dalori, Railway terminus, Gwange bye pass, and from a cattle farm in the outskirts of Molai. Samples were collected in labeled sterile sample bottles, transported in an ice pack and stored at between -8 to 4 °C until analyzed.

SAMPLING TECHNIQUE

Fermented milk was randomly sampled at different milk selling points. A simple random sampling method was employed. Milk vendors and milk maids were interviewed before purchase to avoid buying milk from same source. Raw cow milk samples were collected directly from the udder of nursing cows, at the designated cattle farm or ranches, Table 1.

Table 1: Collection of milk/method of collection

Factors	Frequency N = 172	Percentage (%)
Total Raw Milk (Direct milking output)	86	50
Total Fermented Milk (Milk collected from markets)	86	50

SAMPLE ANALYSIS

Samples were analyzed at the drug analysis laboratory of the National Agency for Food, Drug, Administration and Control (NAFDAC) Area Laboratory, Maiduguri, Borno State, Nigeria using Spectrophotometer Ultra Violet Visible Spectrum UV-VIS (Lambda 35, Perkins Elmer Inc Ltd USA). Method used was according to CODEX Pharmaceutical analysis modern methods (CODEX, 1984).

CHEMICALS AND REAGENTS

Oxytetracycline dehydrate analytical drug standard was obtained from Sigma Aldrich Chemical Co, Steinheim, Germany. Analytic grade Citric Acid, Succinic Acid and di-sodium hydrogen orthophosphate anhydrous (Na_2HPO_4), Sodium Ethylene di-amine Tetra-acetic Acid (Na-EDTA), Potassium Hydrogen Phosphate, Orthophosphoric acid, Hydrochloric (HCl) and Phosphoric acid were all obtained from BDH Chemicals, England.

SAMPLE PREPARATION

Milk samples (raw and fermented) were prepared according to the methods as described by (Samanidou and Nisyriou, 2008). The chemicals prepared and used for the sample clean up include: Mcllvaine-EDTA Buffer (0.1 M sodium EDTA, 0.1 M Citric Acid, 0.2 M di sodium hydrogen phosphate Na_2HPO_4), Citric Acid and 0.1 mol Succinic acid.

MILK SAMPLE CLEAN UP

A 2.5 ml of 0.1 M succinic acid (pH 4) and 5 ml of milk sample was vortexed for 10 seconds. Ten millilitres (10 ml) of Mcllvaine- EDTA (0.1 M sodium EDTA, 0.1 M Citric Acid, 0.2 M di sodium hydrogen phosphate Na_2HPO_4) buffer at pH 4 was also added to the vortexed mixture and was sonicated for 10 minutes and then placed in a freezer for 15 minutes. This mixture was then centrifuged at 4000 rpm at 10°C producing a clear supernatant. The supernatant was filtered with whatman filter paper (110mm Ø) and stored at 4°C until analysis (Samanidou and Nisyriou 2008).

PREPARATION OF STANDARD SOLUTIONS OXYTETRACYCLINE STANDARD PREPARATION METHOD

A 0.1 mol HCl was prepared and was used as the diluent. Five different concentrations (0.01, 0.02, 0.03, 0.04 and 0.05) mg/ml of the oxytetracycline standard were prepared, Table 2.

Table 2: Concentration of Standards prepared for Oxytetracycline

S/N	Concentration of standard (mg/ml)	Absorbance	Wavelength (nm)
1	0.01	0.37	268
2	0.02	0.74	268
3	0.03	1.37	268
4	0.04	1.59	268
5	0.05	1.87	268

PREPARATION OF WORKING CONCENTRATION OF OXYTETRACYCLINE STANDARD

Each of the concentrations of the standards was subjected to analysis in the Spectrophotometer UV-VIS double beam with the diluent in the blank control beam. The absorbencies of all the five different concentrations were recorded after peaking at 268 nm as shown in Table 2. A linear graph of concentration and absorbance was then plotted using excel and the r value determined. r = 0.969, Figure 2.

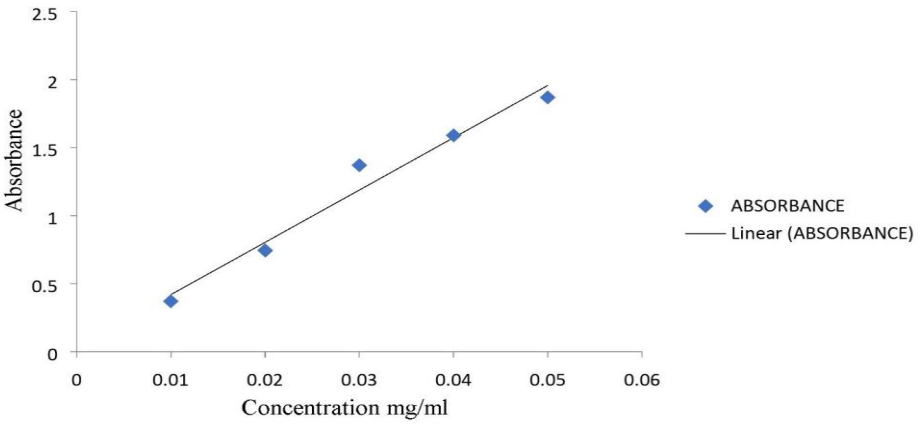

Figure 2: Oxytetracycline analytical standard calibration curve (Linear graph)

From the linear curve in figure, the exponential concentration is 0.04 mg/ml and this was used as the standard working concentration.

EVALUATION OF SAMPLE

An approximately 0.5 ml of standard working concentration (0.04 mg/ml) was pipetted and dispensed into a 10 ml test tube. Two ml of each prepared raw milk sample was added and the solution was topped to 5 ml with the diluent (0.1 mol HCl). This was then transferred into the cuvette and inserted into the machine for analysis. The absorbance of each sample was recorded after peaking at wavelength of 268 nm and concentration of the residue was calculated using the equation from the linear curve according to Beer Lambert's law as thus:

$$y = 38.44x + 0.035$$

Where, y = absorbance and x = concentration.

All the milk samples (86 raw and 86 fermented) were analyzed one after the other for oxytetracycline residue using the same procedure.

PENICILLIN G STANDARD PREPARATION METHOD

Approximately 0.1 mol of HCl was prepared and used as the diluent. Five different concentrations of the penicillin G standard (0.025, 0.05, 0.1, 0.3, and 0.5) mg/ml were prepared, Table 3.

Table 3: Concentration of Standards prepared for penicillin G

S/N	Concentration of standard (mg/ml)	Absorbance	Wavelength (nm)
1	0.025	0.039	257
2	0.050	0.059	257
3	0.100	0.093	257
4	0.300	0.250	257
5	0.500	0.640	257

PREPARATION OF WORKING CONCENTRATION OF PENICILLIN G STANDARD

Each of the concentrations of the standards was subjected to analysis in the UV-VIS double beam with the diluent in the blank control beam. The absorbance of all the five different concentrations were recorded after peaking at 257 nm as shown in Table 3. A linear graph of concentration and absorbance was then plotted using excel and the r value determined. r = 0.949. From the linear curve, the exponential concentration is 0.1 mg/ml and this was used as the standard working concentration, Figure 3.

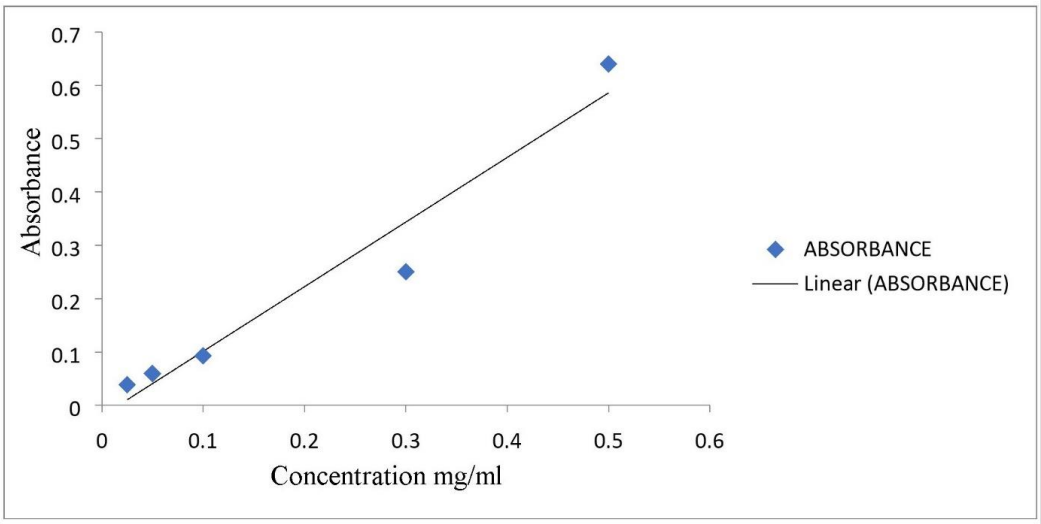

Figure 3: Penicillin G analytical standard calibration curve

EVALUATION OF SAMPLE

An approximately 0.5 ml of standard working concentration (0.1 mg/ml) was pipetted and dispensed into a 10 ml test tube. Afterwards, 2 ml of each prepared raw milk samples was added and the solution was topped to 5 ml with the diluent (0.1 mol HCl). This was then transferred into the cuvette and inserted into the machine for analysis. The absorbance of each sample was recorded after peaking at a wavelength of 257 nm and the concentration of the residue was calculated using the equation from the linear curve according to Beer Lambert's law as thus:

$y = 1.211x - 0.019$ where y = absorbance and x = concentration.

All the milk samples (86 raw and 86 fermented) were analyzed one after the other for Penicillin G residue using the same procedure.

QUANTIFICATION OF OXYTETRACYCLINE AND PENICILLIN G RESIDUE IN MILK SAMPLES (RAW AND FERMENTED)

Extracts of each milk sample (raw and fermented) that was positive as analyzed in the spectrophotometer gave a spectrophotometric peak at a wavelength of 268 nm for oxytetracycline and 257 nm for penicillin G with a retention time of 1 ± 2 minutes for both. This confirmed the presence of oxytetracycline and penicillin G residues. The peak areas of individual samples were recorded and substituted into the linear standard equation to deduce the amount of residue in mg/L (converted to µg/L) present in each sample.

STATISTICAL ANALYSIS

Data obtained in this study was presented in the form of frequency distribution, tables and bar charts. Unpaired sample t test was used to determine differences between means and statistical significance was set at $p \leq 0.05$ using GraphPad Prism (GraphPad 2003), Table 4.

Table 4: Comparison of means between oxytetracycline and penicillin G residue in raw and fermented milk

Groups	Antibiotic residue (µg/L)	
	Oxytetracycline Mean ± SEM	Penicillin G Mean ± SEM
Raw Milk	36.21^{a} ± 2.282	648.7^{c} ± 61.74
Fermented Milk	20.28^{b} ± 0.916	396.5^{d} ± 72.94

Values with different superscripts within rows and columns differ significantly ($p < 0.05$)

RESULTS AND DISCUSSION

LEVELS OF OXYTETRACYCLINE RESIDUE IN FERMENTED MILK IN MAIDUGURI

A total of 86 fermented milk samples were analyzed for oxytetracycline residue and all samples were positive. The concentration of the oxytetracycline in milk samples were compared with the maximum acceptable residue limit as set by CODEX and all samples were below the maximum acceptable residue limit of 100 µg/L, Figure 4.

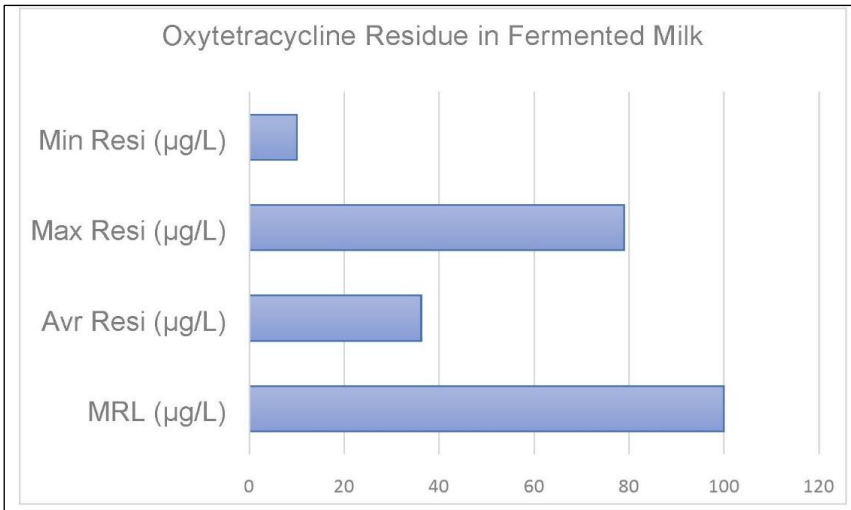

Figure 4: Levels of oxytetracycline residue in fermented milk in Maiduguri

LEVELS OF OXYTETRACYCLINE RESIDUE IN RAW MILK IN MAIDUGURI

A total of 86 raw milk samples were analyzed for oxytetracycline residue and all samples were positive. The concentration of the oxytetracycline in the milk samples were compared with the maximum acceptable residue limit as set by CODEX and all samples were below the maximum acceptable residue limit of 100 µg/L, Figure 5.

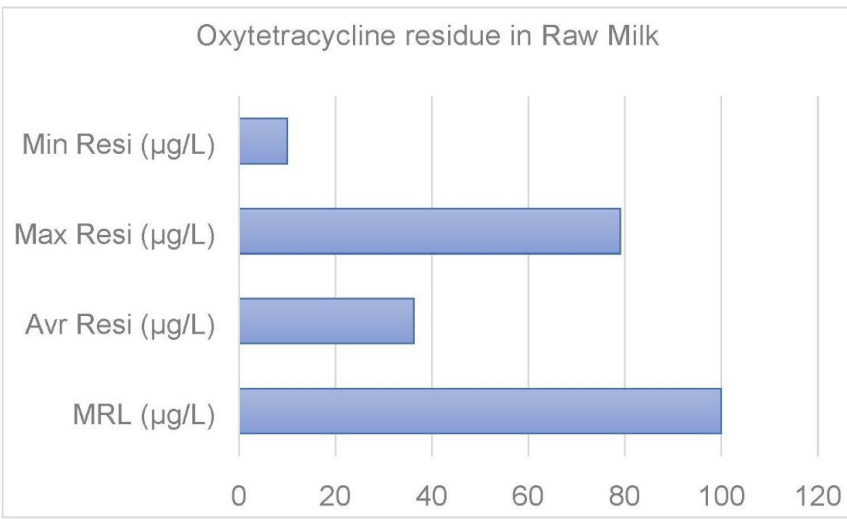

Figure 5: Levels of oxytetracycline residue in raw milk in Maiduguri

LEVELS OF PENICILLIN G RESIDUE IN FERMENTED MILK IN MAIDUGURI

A total 86 fermented milk samples were analyzed for penicillin G residue and all samples were positive. The concentration of the Penicillin G residue in the milk samples were compared with the maximum acceptable residue limit as set by CODEX and all samples were high above the maximum acceptable residue limit of 5 µg/L, Figure 6.

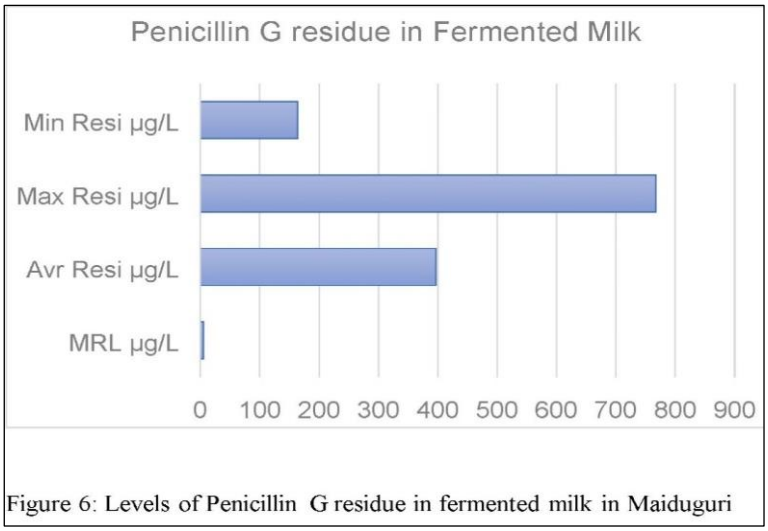

Figure 6: Levels of Penicillin G residue in fermented milk in Maiduguri

LEVELS OF PENICILLIN G RESIDUE IN RAW MILK IN MAIDUGURI.

A total 86 raw milk samples were analyzed for penicillin G residue and all samples were positive. The concentration of the Penicillin G residue in the milk samples were compared with the maximum acceptable residue limit as set by CODEX and all samples were high above the maximum acceptable residue limit of 5 µg/L, Figure 7.

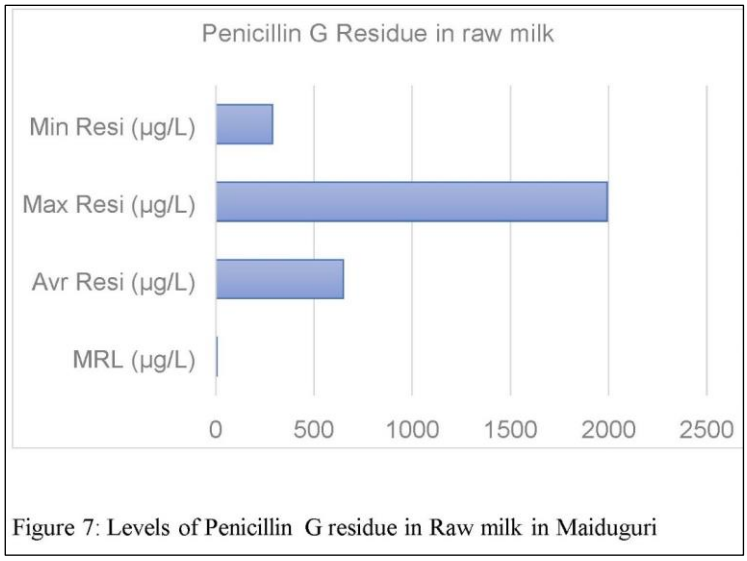

Figure 7: Levels of Penicillin G residue in Raw milk in Maiduguri

DISCUSSION

The findings of this research work revealed that both oxytetracycline and penicillin G (Benzyl Penicillin) antibiotics residues have been detected in all the 172 (86 raw and 86 fermented) milk samples analyzed. Quantitatively, the levels of oxytetracycline detected in all 86 fermented milk samples were below the maximum residue limit of 100 µg/L but penicillin G residues detected in 86 raw milk samples were excessively high above the maximum residue limit of 5µg/L as given by CODEX.

Penicillin G was detected in raw milk at a much higher quantity than others with a mean residue concentration of 649 which is higher than the MRL of 5 µg/L. This finding is similar with the one done by (Kaya and Filazi, 2010). They reported that all samples (240: 120 raw and 120 pasteurized) collected from various markets in Ankara, Turkey contained penicillin residues with a mean concentration of 150.4 µg/L which were higher than the maximum residue limit acceptable in Turkey and other European countries. But this current finding however differs from the one done by (Khaskheli *et al*., 2008) who reported that out of the 137 milk samples screened in Pakistan, 36.05 % were positive for penicillin G residues while 63.50% were negative. The finding also completely differs from another research conducted by Yamaki *et al.*, (2004) in Spain who reported that out of a total 2,686 raw milk samples analyzed, only 1.70% was positive for penicillin.

The result of this current study was also found to be comparable with that conducted by Abebaw *et al*., (2014); Kabrite *et al.,* (2019) who reported an 83.3% and 86.4% prevalence of penicillin G residues in milk samples analyzed in Ethiopia and Lebanon respectively. However, this finding was found to be different from the one conducted by (Olatoye *et al*., 2016) in Osun, Nigeria who reported 41.10% prevalence for penicillin G in raw milk. They further stated that out of the detected residue, 39.30% were above the recommended CODEX maximum residue limit. The next highest antibiotic residue detected was Penicillin G in fermented milk with 100% prevalence and a mean residue concentration of 397 µg/L which is extremely high and above the Maximum residue level of

5 µg/L set by CODEX. This finding differs from the one conducted by (Olatoye *et al.,* 2016) in Osun, Nigeria. They reported a 40.20% prevalence of penicillin G in fermented milk out of which 36.70 % were above the MRL as set by Codex.

The third highest amount of residue detected was oxytetracycline in raw milk which was also 100% positive with mean residue concentration of 36.2 µg/L. However, the residues were all below the maximum recommended residue limits of 100 µg/L set by CODEX. This finding is similar with the one conducted by Tona and Olusola (2014), who reported that all 40 dairy products sampled in Ogbomosho, south western Nigeria, contained residues of tetracycline antibiotics which were all below the MRL. Likewise, Yusuf *et al*., (2017) sampled milk from 54 farms in Kano, Nigeria for the presence of antimicrobial residues and found out that out of 313 milk samples analysed, 25 % were positive for antimicrobial residues, with drugs belonging to tetracycline group having the higher percentage (93.7 %) and all were below the MRL. However, the current finding is different with the one by Navrátilová *et al.,* (2009) who examined bulk milk (n = 57) and tanker trailer (n = 113) samples in Czech Republic and reported that residues of tetracycline and oxytetracycline were detected in 50.6% of analyzed samples.

The least mean residue concentration 20.3µg/L was oxytetracycline in fermented milk which was also far below the MRL of 100 µg/L set by CODEX. This finding is in line with the one conducted by Zhang *et al.,* (2014); Kabrite *et al*., (2019). They examined pasteurized milk to verify the presence of residue of tetracycline in China and Lebanon and none exceeded the MRL established by CODEX.

The presence of high concentration of penicillin G residue in raw milk might be connected with the fact that farmers and herdsmen mostly use injectable penicillin preparations intra mammary or systemically for the prevention or treatment of mastitis in lactating cows. This trend has been in practice for a long period of time (Pyorala, 2009; Ogunshe and Adeola, 2019; Mangesho *et al.,* 2017).

Unfortunately, all the clinical implications such as alteration of the delicate balance of intestinal flora, mutagenicity, hepatotoxicity, bone marrow toxicity, carcinogenicity and antibiotic resistance (Beyene, 2016) were very unclear to the cattle farmers and traders, not only because they cannot understand the implications of such unhealthy activity, with regards to zoonosis, but because their major aim is to maximize profits. Furthermore, the high presence of penicillin residue in raw milk might also be linked to the fact that farmers and herdsmen usually dose their cows with these chemotherapeutic agents immediately after parturition to prevent or treat post partum complications or mastitis (Priyanka *et al.,* 2017)

The second-high concentration of penicillin residue was detected in fermented milk. The low levels of these antibiotic residues in fermented milk compared to raw milk might be due to the effect of heat on processing (Vivienne *et al*., 2018)

CONCLUSION

Prevalence and high amount of penicillin G residues in raw and fermented milk samples sold in Maiduguri, North Eastern Nigeria as discovered in this current study is not surprising because there has not been any monitoring programme put in place by government neither has there been a deliberate effort to sensitize the populace on the dangers associated with residues in animal products. The lack of the enforcement of regulatory laws on veterinary drug use in Nigeria (Nisha, 2008; Dina and Arowolo, 1991) has led to the abuse and misuse of drugs in food animals. This is done through indiscriminate use of this chemotherapeutic agents for prevention and treatment of diseases in dairy farms, treatment of diseased cows by owners without professional advice, poor knowledge about drug withdrawal periods (Jayalakshmi *et al.,* 2017), lack of proper management of dairy cows and poor awareness of the people concerning health impact of antibiotic residue in milk were the major contributing factors (Singh *et al.,* 2014).

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Conflicts of Interest: The authors declared that there is no conflict of interest whatsoever with this work.

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