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Original Research Article

Detection of Oxytetracycline Residues in Table Eggs in Khartoum State

ABSTRACT

Aims: This study was conducted to detect the presence of oxytetracycline residues in table eggs in Khartoum State and to compare its level with the international acceptable maximum residue limits (MRLs).

Study design: One hundred and eighty table egg samples were randomly collected from 18 sale points in the three localities of Khartoum State, (60 eggs from Omdurman, 60 eggs from Khartoum and 60 eggs from Khartoum North).

Place and Duration of Study: samples collected from Khartoum State during August and September, 2015.

Methodology: Microbiological inhibition assay was used to screen the presence of antibiotic residues using Bacillus subtilis seeded in nutrient agar. Ninety positive egg samples resulted from microbiological inhibition assay were analyzed to detect the presence and quantify oxytetracycline residues by using HPLC.

Results: Microbiological inhibition assay showed that 50% of the tested samples were positive for antibiotic residues in Omdurman, Khartoum and Khartoum North with percentages 18.9%, 15.6%, and 15.6%, respectively. HPLC results showed that 70% were positive for oxytetracycline residues above the permissible maximum residue limits in the16 sale points in Omdurman and Khartoum, Khartoum North and Omdurman with percentages of 21.2%, 23.3%, and 25.5% respectively.

Conclusion: It was concluded that high percentage of table eggs contained oxytetracycline residues above the maximum residue limits that indicated the widespread misuse of oxytetracycline in poultry farms that may cause health hazards to the consumer in Khartoum State. Therefore the study recommends compliance of drug withdrawal periods in poultry farms could reduce the incidence of antibiotic residues in consumed eggs.

(*Keywords: oxytetracycline. residue, table eggs, health, microbiological, HPLC, analysis)*

UNDER PEER REVIEW

1. INTRODUCTION

Birds are reared for meat and egg production as an important protein sources. Drugs are an essential part of poultry production and used to prevent and control diseases, reduce stress due to environmental changes, vaccination and other management practices [1]. Recently in Sudan, the poultry industry is largely increasing. The use of antibiotic as a routine practice is non-avoidable to prevent economic loss due to diseases and consequent mortality [2].

The frequent use of antibiotics in clinical practice causes the occurrence of antibiotic residues in various food products of animal origin including milk, egg, and meat [3].The presence of antibacterial drug residues in eggs can cause health hazards for consumers like hypersensitivity reaction, development of resistant organisms to these antibacterial agents and destruction of gastrointestinal natural flora[4].The consumer in Khartoum State is at constant risk of exposure to antimicrobial residues in table eggs[5]. Oxytetracycline (OCT) is a broad spectrum antibiotic against Gram-positive and Gram-negative bacteria. It is used for the treatment of bacterial diseases and most commonly applied among tetracycline group for food producing animals. OTC also finds application in human therapy [6]. It's widely administered to farm animals for the purpose of therapeutically treatment and health protection [7]. Worldwide, national and international public health agencies have a deep concern about the presence of antibiotic residues in meat, egg and edible viscera of food producing animal [8, 9]. The maximum residue levels (MRLs) of antibiotics for veterinary drugs are implemented by the European Union and similarly suggested by other international institutions to prevent these problems and safeguard consumers' health [10, 11]. Oxytetracycline residue was detected in table eggs in study done by Alghamdi [12] Naser [13] European Union has established the maximum residual limit (MRL) of oxytetracycline in eggs at 0.2ppm [10].

2. MATERIAL AND METHODS

2.1Sample collection

A total of 180 table eggs samples were collected randomly from 18 sale points in the three major localities in Khartoum State, (60 eggs from Omdurman, 60 eggs from Khartoum North, 60 eggs from Khartoum) during August and September, 2015. The source of samples in the sale points are 18 farms.

UNDER PEER REVIEW

2.2 Sample handling and processing

The eggs samples were transported to the Central Veterinary Research laboratory in carton and plastic storage trays at room temperature and processed within 24 hours post collection. In a cabinet under septic condition the surface of each egg sample was disinfected using sterile cotton soaked in 70% ethyl alcohol. A small opening was made at the tip of the egg using sterile thump forceps then the albumen and yolk were mixed carefully using a sterile cotton swab, then removed into a sterile universal container and preserved at -20°C until being analyzed.

2.3 Evaluation of antibiotic residues

Two methods were used simultaneously for the determination of oxytetracycline residues in table eggs namely screening test that include microbiological inhibition assay and confirmatory test using high performance liquid chromatography (HPLC).

2.3.1 Microbiological inhibition assay

Bacillus subtilis (ATCC6633) was obtained from the Department of Bacteriology, Central Veterinary Research Laboratory and used as indicator organism according to the method described by Ellerborek [14] with some modification.

2.3.1.1 Preparation of Bacterial suspension

Aseptically some colonies of *Bacillus subtilis* previously grown on nutrient agar slant, were taken by a sterile loop, cultured onto nutrient agar plate then incubated at 37°C for 18hours. One colony from the fresh culture was taken by a sterile loop then diluted in 5ml sterile 0.85% NaCl the suspension was mixed by vortex mixer then adjusted to 0.5 McFarland tube.

2.3.1.2 Preparation of culture medium

Nutrient agar medium (Oxoid) was prepared according to the manufacture instructions then cooled to 45-50 °C. 10microlitres of the bacterial suspension was taken by micro pipette and inoculated into the medium then mixed gently and poured into sterile Petri dishes in 25ml volume and left to solidify.

2.3.1.3 Test procedure

UNDER PEER REVIEW

Four wells were made in each petri dish by a sterile puncher. 0.25g from the albumin and yolk mixture was taken by a sterile syringe, inoculated into the wells then incubated aerobically at 37°C for 18hours. The presence of antibiotic residues in the sample was indicated by the appearance of inhibition zone around the wells.

2.3.2 .HPLC analysis

HPLC analysis was used for the determination of oxytetracycline residues level in 90 positive samples resulted from the microbiological inhibition assay.

2.3.2.1 Materials

Oxytetracycline and tetracycline reference standards were obtained from Dopine Department, Pendik Institute, (Republic of Turkey), Citric acid, HPLC grade acetonitril (ACN) and methanol (MEOH) HPLC Grade were obtained from Sharlau (Germany), nitric acid 30%, deionized water and sulfuric acid.

2.3.2.2 Preparation of oxytetracycline standard stock and working solutions

Stock solution of 1mg/ml of oxytetracycline was prepared by dissolving0.01g of oxytetracycline standard in100ml methanol HPLC grade in volumetric flask. The working solutions of 200, 100, 50, 25 µg/ml was prepared by addition of 200, 100, 50, 25 µl of stock solution methanol to be completed to one ml each concentration respectively.

2.3.2.3 Preparation of tetracycline standard stock

Stock solution of 1mg/ml of tetracycline standard was prepared by dissolved tetracycline in methanol then preserved at 4°c in a fridge. Tetracycline was used as internal standard.

2.3.2.4 Preparation of mobile phase

Fifteen ml of Acetonitrile (ACN) was added to 85 ml of deionized water (pH = 2.1) with sulfuric acid (15:85) completed 100ml. fresh mobile phase was prepared daily before work.

2.3.2.5 Sample extraction

Eggs samples were extracted by liquid – liquid extraction according to method described by Senyuva [15] .Two gram of homogenized egg sample were taken and transferred into centrifuge tube, 100µg/ml of tetracycline,

UNDER PEER REVIEW

0.1 g citric acid, 1ml nitric acid (30%), 4 ml methanol and 1 ml deionized water were added respectively. The suspension was vortexed, kept in ultrasonic water bath for 15 min and then centrifuged at 5300 rpm for 10 min. the supernatant was taken and filtered through a 0.22 µm syringe filter then 20 µl of solution was injected into HPLC for analysis.

2.3.2.6 Preparation of standard curve

Calibration standards were prepared using concentrations of 25, 50, 100 and 200 µg/ml of OTC. Four tubes were labeled as 25, 50, 100 and 200 µg/ml. two gram of blank egg sample was added to each tube. Each tube was vortexed to 30scends then the samples were extracted by the same procedure described previously.

2.3.2.7 HPLC determination of oxytetracycline residue concentration

Determination of oxytetracycline residues was performed by using a HPLC system(Cykam,Germany) and S3210 uv-vis detector, Cykam S1122 quaternary pump and column type Interstil ODS-3 C18(5 µm, 150 X 4.6 mm). The detecting wavelength was set at 360 nm and personal computer software (simple peak) was used for analyzing data. The mobile phase contained deionized water (pH = 2.1 with sulfuric acid) (A) and ACN (B). Isocratic solvent program was run (85 %(A): 15 % (B), v/v). The Flow rate was 1.5 ml/min. HPLC analysis of the samples was performed in 10min. Peak area ratios of oxytetracycline were converted in to concentrations using calibration curve.

3. RESULTS AND DISCUSSION

3.1 Microbiological inhibition assay

Of the180 egg samples 90(50%) showed the presence of antibiotic residues and 90(50%) were negative. The total number of positive and negative samples was found in collected samples from Omdurman, Khartoum and Khartoum North are shown in Figure 1.

UNDER PEER REVIEW

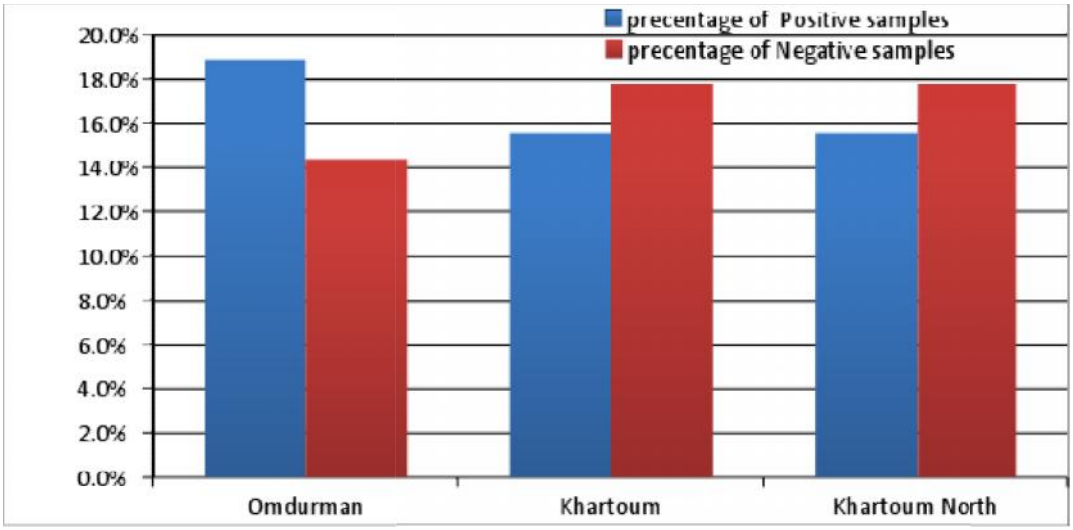

Figure 1: Percentage of positive and negative eggs samples for antibiotics residues using microbiological inhibition assay in Omdurman, Khartoum and Khartoum North.

3.2 HPLC analysis

Of the 90Positive sample for antibiotic residues in Khartoum State showed that 63 (70%) were positive for oxytetracycline residues above the permissible maximum residue limits and 27(30) % were negative (Figure 2).Mean concentration of oxytetracycline residues in positive eggs were found in Omdurman 24.1 ppm, Khartoum 24.7 ppm and Khartoum North 30.6ppm.

3.2.1 Retention time of oxytetracyline and tetracycline

Retention time of oxyteracyline and tetracycline were indicated by the appearance of peak after injecting different concentrations of standards in HPLC. The retention time of oxyteracyline and tetracycline were found 4.4-7.4min and 6-7min respectively.

3.2.2 Standard curve

The standard curve showed good linearity over the range of concentrations examined (25-200µg/ml) where a straight line passing through the origin with a correlation coefficient of R2 =0.9965 was obtained for OCT. (Figure 3).

UNDER PEER REVIEW

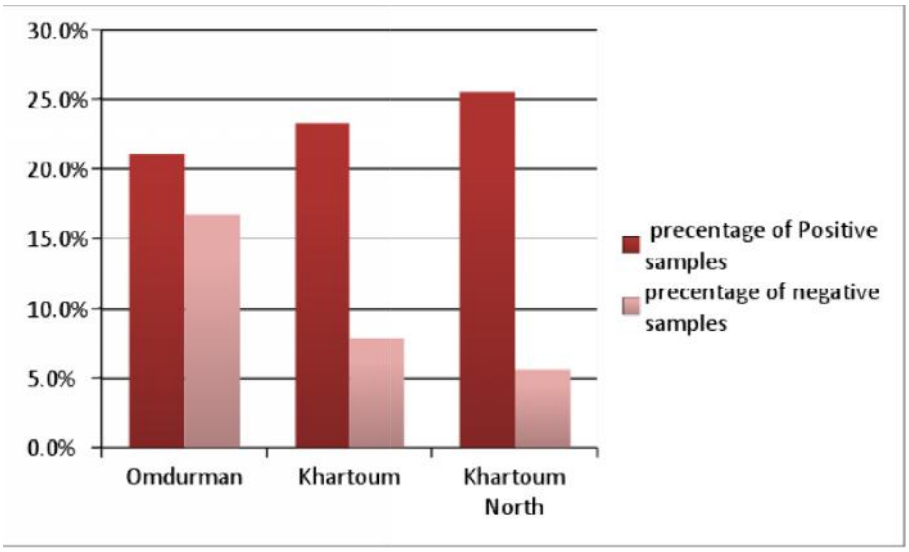

Figure 2: The percentage of positive and negative eggs samples for oxytetraycline residues using HPLC in Omdurman, Khartoum and Khartoum North.

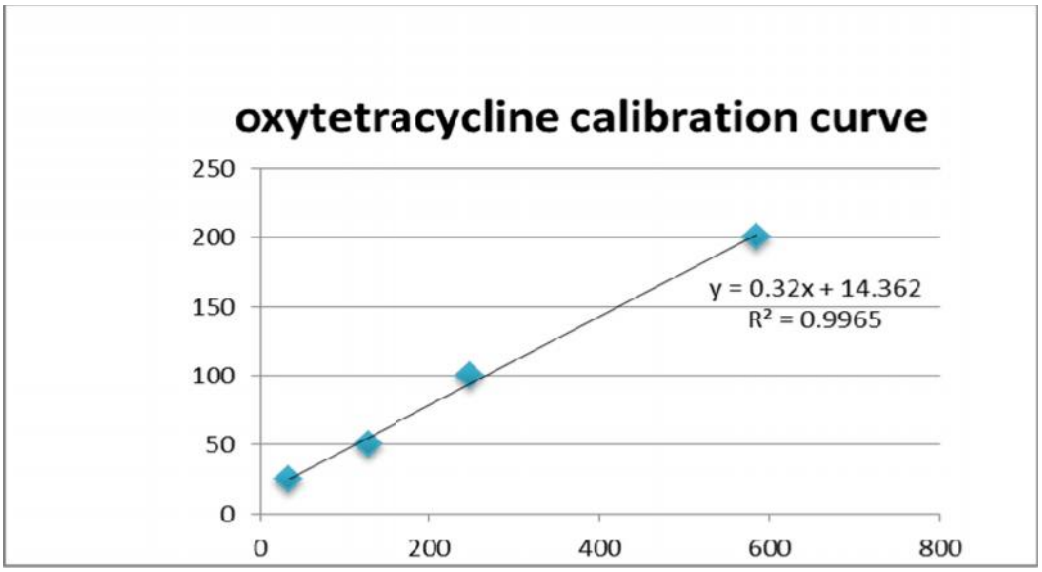

Figure 3: 0xytetracycline calibration curve.

Table eggs in Khartoum State were screened for the presence of oxytetracycline residues by using microbiological inhibition assay and Hplc, The microbiological method can detect a wide range of antibiotics thresholds close to the maximum residue limits (MRLs) [1 ,16]. The results of present study revealed that 50% of egg samples were positive for antibiotic residues in 16 sale points in the three localities in Khartoum State. Higher percentages of positive samples for antibiotic residues were observed in Omdurman 18.9%, Khartoum

UNDER PEER REVIEW

15.6%, and Khartoum North 15.6%. Nearly similar results reported by study done in Khartoum State [17, 5]. Our results showed that table eggs in sale points were contaminated with antibiotic residues that may expose consumers to health hazards and also indicated the widespread misuse of antibiotics may be as a result of poor hygienic status of poultry farms with the continuous use of antibiotics for treatment of diseases and prophylaxis purpose. It was also proposed that lack of knowledge about withdrawal period regarding the use of antibiotics resulted in the high percentage of positive samples [17].

Positive samples were analyzed for the detect and quantify oxytetracycline residues by using high performance liquid chromatography method and results revealed that 70% were positive for oxytetracycline residues above the permissible maximum residue limits in16 sale points in three localities in Khartoum State. Higher percentages of positive samples for antibiotic residues were observed in Khartoum North 25.5% Khartoum 23.3% Omdurman 21.1%. Some positive samples for microbiological inhibition assay showed negative results for oxytetracycline residues may be due to variation of antibiotic use by different farms. This was in line with other investigators (Hind *et al*., 2012) who reported that the most frequently used antibiotic was tetracycline in 36.4% of the farms. Nearly findings reported by Alghamdi *et al.,* (2000) who found higher usage of tetracycline in eggs in Saudi Arabia using the high-performance liquid chromatography (HPLC) and Naser, (2012) who found positive samples with seasonal difference in different area .These results showed a strong contamination of the table eggs samples for oxytetracycline residues that may indicate there was wide use for oxytetracycline and widespread misuse by egg layer breeders in Khartoum State.

4. CONCLUSION

This study concluded that high percentage of the table egg samples in sales Point Khartoum State contained oxytetracycline residues above the MRL that may indicate the widespread misuse of oxytetracycline in poultry farms which can result in serious health hazard.

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UNDER PEER REVIEW

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UNDER PEER REVIEW

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