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Detection of Oxytetracycline Residues in Table Eggs in Khartoum State, Sudan

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Authors' contributions

This work was carried out in collaboration between all authors. Author EAH designs the study, wrote the protocol and the first draft of the manuscript and conduct the laboratory analysis of the samples. Author KMO supervised design the study and managed the literature searches. Author IGI performed the statistical analysis and supervised the quantitative analysis. Author YAS supervised the qualitative analysis of the sample and revised the manuscript. All authors read and approved the final manuscript.

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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, Sudan, (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 from the microbiological inhibition assay were analyzed to detect the presence and quantity of oxytetracycline residues 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 34(18.9%), 28(15.6%), and 28(15.6%) of the antibiotics respectively. HPLC results showed that 63(70%) were positive for oxytetracycline residues 19(10.5%) from Omdurman, 21(11.6%) from Khartoum and 23(12.7%) from Khartoum North.

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Conclusion: It was concluded that high percentage of table eggs contained oxytetracycline residues above the MRLs (0.2 ppm) that indicated the widespread misuse of oxytetracycline in poultry farms that may cause health hazards to consumers 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.

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 has been growing. The use of antibiotics in routine poultry production has become unavoidable in order to prevent economic losses due to poultry diseases [2].

The frequent use of antibiotics for livestock disease prevention 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 pose a health hazard to consumers such as hypersensitivity reaction, development of resistant organisms to these antibacterial agents and destruction of gastrointestinal natural microbiota [4]. Consumers in Khartoum state constantly face the 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 tetracyclines group for food-producing animals. OCT also finds application in human therapy [6]. It is widely administered to farm animals for the purpose of health protection and prevention of diseases [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 animals [8,9]. The maximum residue levels (MRLs) 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 residues were as detected in table eggs in studies conducted by Alghamdi [12] and Naser [13]. The European Union has established the maximum residue limit (MRL) of oxytetracycline in eggs at 0.2 ppm [10]. This study was aimed 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).

2. MATERIALS AND METHODS

2.1 Sample Collection

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

2.2 Sample Handling and Processing

The egg samples were transported to the Central Veterinary Research laboratory in carton and plastic storage trays at room temperature (22-25°C) and processed within 24 hours post collection. The surface of each egg was disinfected using sterile cotton soaked in 70% ethyl alcohol in a cabinet (under septic conditions). A small crack was made at the tip of the egg using a sterile thump forceps, after which the albumen and yolk were carefully mixed using a sterile cotton swab, which was subsequently removed into a sterile universal container and preserved at -20°C until analysed.

2.3 Evaluation of Antibiotic Residues

Two methods were used simultaneously for the determination of the presence oxytetracycline residues in table eggs namely screening test that included microbiological inhibition assay and confirmatory analysis 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, Khartoum, Sudan and used as an indicator organism according to the method described by Ellerbrock [14] with some modifications'.

2.3.1.1 Preparation of bacterial suspension

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

2.3.1.2 Preparation of culture medium

Nutrient agar medium (Oxoid) was prepared according to the manufacturer's instructions then cooled to 45-50°C. 10 µL (1.5×10^6 CFU) of the bacterial suspension was taken by micropipette 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

Four wells were made in each Petri dish by a sterile puncher. 0.25 g from the albumin and yolk mixture was taken using a sterile syringe, inoculated into the wells then incubated aerobically at 37°C for 18 hours. 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 levels in 90 samples that tested positive using the microbiological inhibition assay.

2.3.2.1 Materials

Oxytetracycline and tetracycline reference standards were obtained from Doping Department, Pendik Institute (Republic of Turkey). Citric acid, HPLC grade acetonitrile (ACN) and methanol were obtained from Sharlau (a company in Germany), nitric acid 30%, deionised water and sulfuric acid were obtained from Sharlau (the company in Germany).

2.3.2.2 Preparation of oxytetracycline standard stock and working solutions

A stock solution of 1 mg/ml of oxytetracycline was prepared by dissolving 0.01 g of oxytetracycline standard in 100 ml methanol HPLC grade in a volumetric flask. The working solutions of 200, 100, 50, 25 µg/ml were 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

The stock solution of 1mg/ml of tetracycline standard was prepared by dissolving 0.01g tetracycline in 100 ml methanol then preserved at 4^oC 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 deionised water (pH = 2.1) with sulfuric acid (15:85) made up to 100 ml. Fresh mobile phase was prepared daily before work.

2.3.2.5 Sample extraction

Egg 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 the centrifuge tube, 100 µL (100 µg/ml) of tetracycline was added as internal standard, 0.1 g citric acid, 1 ml 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 Validation of the method

Calibration standards were prepared using concentrations of 25, 50, 100 and 200 µg/mL of oxytetracycline. Drug spiked egg sample were prepared by adding appropriate amount of stock solution to two gram of blank egg sample to make a final concentration of 25, 50, 100 and 200 µg/kg. The precision of the method was evaluated by calculating relative standard deviation (RSD) in intraday repeatability. RSD calculated from three replicates for each spiking level.

2.3.2.7 HPLC determination of oxytetracycline residues concentration

Determination of oxytetracycline residues was performed by using an 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 to concentrations using the calibration curve.

2.4 Statistical Analysis

One- sample T-test was used to study the statistical association between the different concentration of oxytetracycline residues in compare with maximum residues limits. One-way ANOVA was used to compare with varying levels of oxytetracycline residues in different areas using IB M SPSS statistics 20.

3. RESULTS AND DISCUSSION

3.1 Microbiological Inhibition Assay

Of the 180 egg samples, 90 (50%) showed the presence of antibiotic residues and 90 (50%) were harmful. The total number of positive and negative samples which were found in samples collected from Omdurman, Khartoum and Khartoum North are shown in Fig. 1.

3.2 HPLC Analysis

Of the 90 Positive samples for antibiotic residues in Khartoum State, showed that 63 (35%) were positive for the residues above the permissible maximum residue limits and 27 (15) % were below (Fig. 2). The mean concentration of oxytetracycline residues in positive eggs were 24.1 ± 3.37 ppm in Omdurman, 24.7 ±4.25ppm in Khartoum and 30.6±9.34 ppm in Khartoum North respectively with significant difference $P<0.05$ in contrast maximum residues limit. There is no significant difference $P>0.05$ in concentration of oxytetracycline residues in different areas.

3.2.1 Result of validation of method

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 R^2; 0.9965 was obtained for oxytetracycline (Fig. 3). All RSD % value for intraday repeatability ranging from 1.40 - 7.40. Trueness' expressed as relative recovery 73.9 - 92%.

Table eggs in Khartoum State were screened for the presence of oxytetracycline residues by using microbiological inhibition assay and HPLC. The microbial method can detect a wide range of antibiotics thresholds close to the maximum residue limits (MRLs) [1,16]. The results of the present study revealed that 50% of egg samples

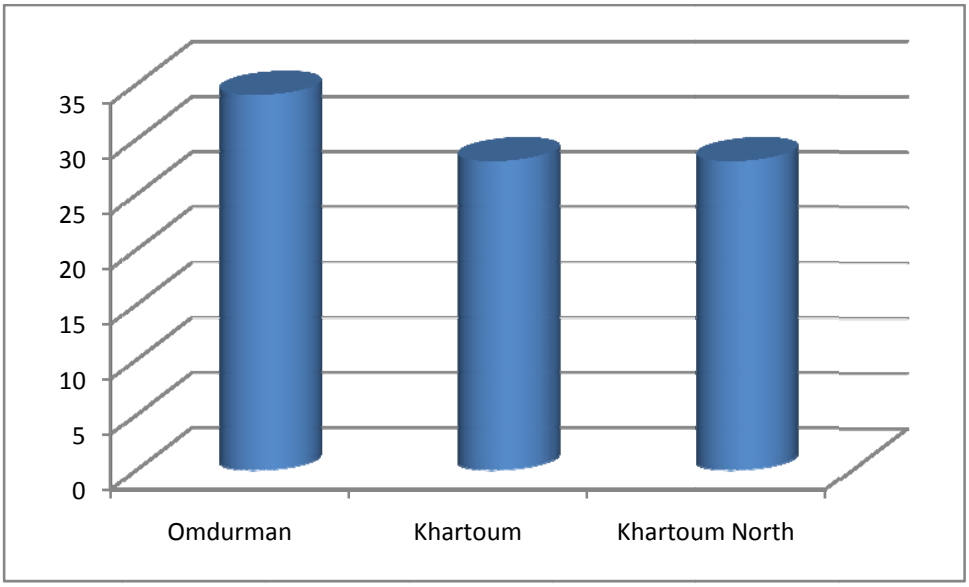

Fig. 1. The number of positive egg samples for antibiotics residues using microbiological inhibition assay in Omdurman, Khartoum and Khartoum North

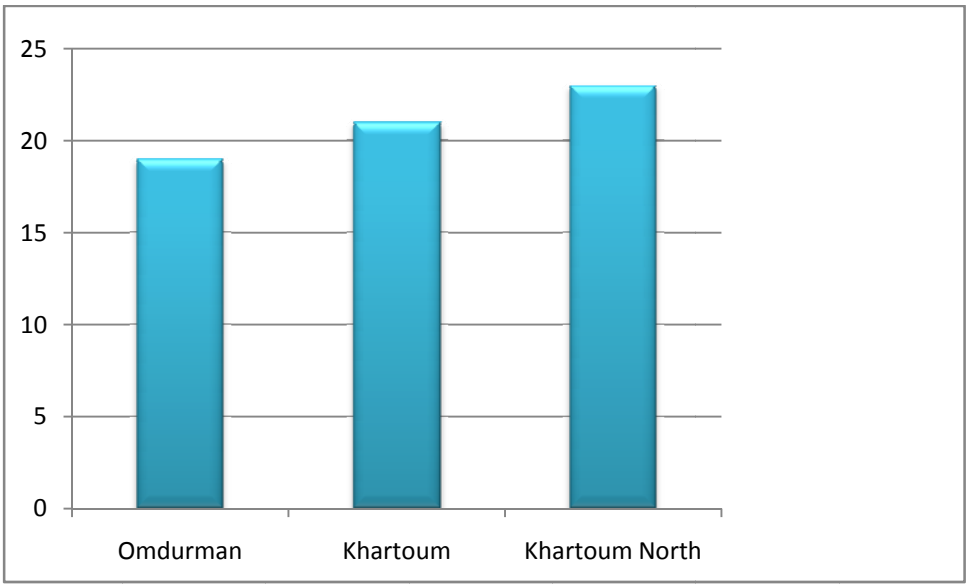

Fig. 2. The number of positive egg samples for oxytetracycline residues using HPLC in Omdurman, Khartoum and Khartoum North

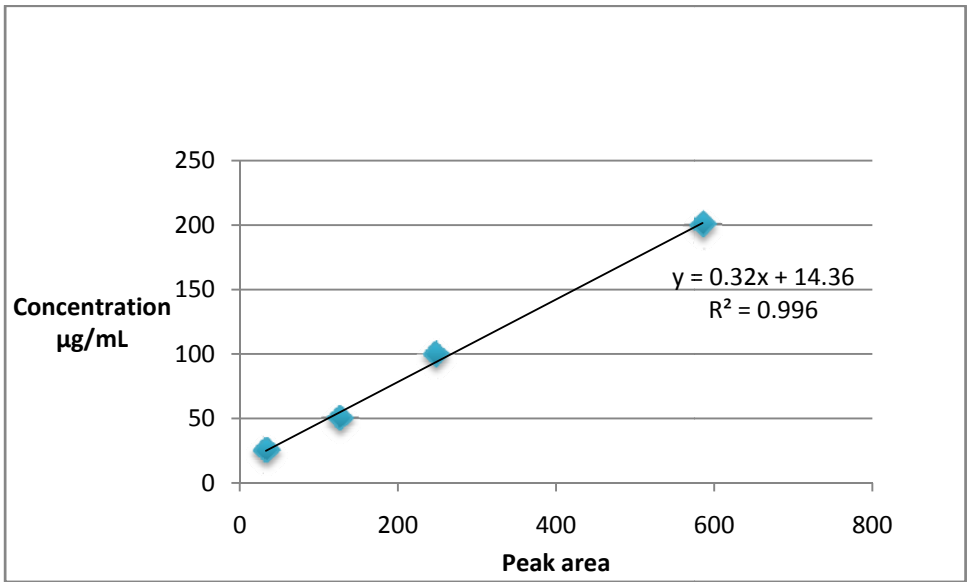

Fig. 3. Oxytetracycline calibration curve

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% than Khartoum 15.6% and Khartoum North 15.6%. Nearly similar results were reported in a 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. This also indicated the widespread misuse of antibiotics which may be as a result of the poor hygienic status of poultry farms necessitating the continuous use of antibiotics for treatment of diseases and prophylaxis purpose. It was also proposed that lack of knowledge about observing the withdrawal period following the use of antibiotics resulted in the high percentage of positive samples [17]. Samples positive for drug residues were further analyzed for the detection and quantification of oxytetracycline residues using high performance liquid chromatography method and results revealed that 63(35%) were positive for oxytetracycline residues above the

permissible maximum residue limits according to (European Union Regulation, 2009) in the 16 sale points in three localities in Khartoum State. Higher percentages of positive samples for antibiotic residues were observed in Khartoum North 12.7% Khartoum 11.6% Omdurman 10.5%. Some positive samples for microbiological inhibition assay showed negative results for oxytetracycline residues for HPLC might be due to the 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. The observations in the present study are similar to the findings published by Alghamdi et al. (2000) who found the 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 differences in different areas. These results showed substantial contamination of the table eggs with oxytetracycline residues that may indicate extensive usage for oxytetracycline and widespread misuse by breeders (poultry) in Khartoum State.

4. CONCLUSION

In conclusion, this study has shown that a high percentage 63(35%) of table eggs from sale points in Khartoum State contain oxytetracycline residues above the MRL that may indicate the widespread misuse of oxytetracycline in poultry farms which can result in serious health hazards. Therefore the poultry farms must be kept under the veterinary supervision and Adherence to good veterinary practices, such as layer hens hygiene, immunisation and selection of farms free of bacteria. Control of using oxytetracycline for treatment or as feed additives and excellent observation of withdrawal times after oxytetracycline treatment can decrease the incidence of oxytetracycline residues in table eggs. Eggs collected during and shortly after antibiotic medication should not be used for human consumption.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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