# Determination Of Some Antibiotics And Sulphonamide Residues In Meat And Liver Of Rabbit Carcasses

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**ABSTRACT**

A total of forty samples of muscle and livers of slaughtered rabbits were collected from poultry shops at Tanta city, Gharbia governorate. They were examined for presence of oxytetracycline, ampicilin and sulfadimidine residues using four plate test (FPT) and high performance liquid chromatography (HPLC). The results showed that 5% and 25% of muscles samples were positive for the presence of oxytetracycline and sulfadimidine residues, respectively detected by FPT, while 20%,15% and 45% of liver samples were positive for presence of oxytetracycline, ampicilin and sulfadimidine, respectively. The current results indicated that the incidence of oxytetracycline, ampiciilin residues were 10%, while sulfadimidine was 40% in muscles samples, While the mean value for liver samples was 30%, 20% and 65% for oxytetracycline, ampicilin and sulfadimidine detected by HPLC., respectively. Moreover, 20% of liver samples exceeded the safe limit of oxytetracycline, 5% and 15% of muscles and liver samples respectively exceeded the safe limit of ampicilin while 25% and 55% of the same samples exceeded the safe limit of sulfadimidine residues, respectively. The public health significance of antibiotics and sulfadimidine residues as well as the suggestive recommendations to avoid the presence of such residues were discussed.

## INTRODUCTION

Antibiotics are widely used in food-producing animals for treatment of diseases and as dietary supplements. They may be administered orally as food additives or directly by injection. The use of antibiotics may lead to drug residues in the meat, especially if they are not used according to label directions. The presence of antibiotic residues in meat, milk, etc. may cause allergic reactions in sensitive individuals *(1)*.

Residues of veterinary drugs in food of animal origin represent risks of direct and indirect jeopardy to human health, and they have a negative impact on technological processes in the food industry. From the point of view of the threat to consumer health, antibiotics used in animals are included in the food chain and cause the creation of resistance and allergy *(2,3)*.

Antibiotics may interfere with further food processing if it depend on fermentation reaction. They may cause allergic reactions in highly sensitized consumers. A small numbers of antimicrobials are suspected of having carcinogenic properties *(4)*.

A number of possible adverse health effects of veterinary drug residues have been suggested. These include allergic/toxic reactions to residues, chronic toxic effects occurring with prolonged exposure to low levels of antibiotics, development of antibiotic-resistant bacteria in treated animals. These bacteria might then cause difficult-to-treat human infections and disruption of normal human flora in the intestine *(5)*.

There is a paucity of information on antibiotic and sulphonamide residues in rabbit meat and its organs in Egypt .Therefore, the purpose of this study was to assess antimicrobial residues in rabbit meats and liver qualitatively by use FPT and quantitavely by HPLC.

## MATERIAL AND METHODS

A total of 40 samples of muscle and liver (20 of each)were collected from 20 rabbit carcasses after being dressed and purchased at different poultry shops fromTanta city .Each

sample was individually wrapped in a poly ethylene bag and analyzed for the detection of some antibiotics (oxytetracyclene and ampicillin) and sulfonamides (sulfadimidine) residues either by FPT or HPLC techniques.

**1.Four Plate Test "FPT" (Microbiological method):**

The method recommended by ***Bogaerts and Wolf (6)*** was carried out. Each sample was divided and applied to four plates of antbiotic agar medium; three of wich were inoculated with *bacillus subtilis* spores at pH 6, 7.2 and 8. Moreover, trimethoprim was incorporated into the medium at pH 7.2 to enhance the test for sulfonamide residues. Diffusion of the active oxytetracyclene, ampicillin and sulfadimidine residues was detected by the formation of inhibition zones on one or more plates after incubation at 37° C overnight. The sensitivity of the test was monitored by applying of 6 mm diameter discs containing standard quantities of known antimicrobial agents in each run.

**2.Application of HPLC technique**

Quantitative analysis of antimicrobial agents in the examined samples of rabbit muscle and liver samples were done *(7,8)*.Accurately, 5 grams from each sample and 10 grams of anhydrous sodium sulfate were blended with 20 ml of ethyl acetate in a blender and then centrifuged. The supernatant was evaporated to dryness under reduced pressure at 40° C .The residue was dissolved in 5ml of ethyl acetate-n-hexane (50%) and the solution was applied to Elute previously washed by 5 ml n- hexane. The cartridge was washed with 3ml n- hexane and air dried by aspiration. The antimicrobial residues survey was eluted from the cartridge with 5 ml acetonitrile (20%) and 0.05M ammonium format. The preparation was injected into HPLC system (model LC-10A series equipped with constant flow pump and variable wave length U/V detector, Kyoto, Japan).

Accordingly, oxytetracyclene, ampicillin and sulfadimidine residues were estimated using their standard solutions specific for each of them. Operating conditions for analysis of oxytetracyclene and ampicillin were elutant at 35° C;flow rate 1ml/minute , injection volume, 10µl;detection wave length 216nm.while ,the operating conditions for analysis of sulfadimidine were : eluant at 30° C; flow rate 1ml/minute; injection volume ,20µl and detection wave length272nm.

Statistical analysis of the obtained results was done by application of student " t-test" *(9)*.

## RESULTS

Table 1. Incidence of antibiotic and sulfadimidine residues in rabbit muscle and liver samples by using of (FPT) and HPLC methods (n = 20)

| Antibacterial residues | FPT * Muscle samples | | HPLC** Muscle samples | | FPT Liver samples | | HPLC Liver samples | |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % |
| Oxytetracyline | 1 | 5 | 2 | 10 | 4 | 20 | 6 | 30 |
| Ampicillin | - | - | 2 | 10 | 3 | 15 | 4 | 20 |
| Sulfadimidine | 5 | 25 | 8 | 40 | 9 | 45 | 13 | 65 |

No = number of positive samples.

*FPT= Four plate test as microbiological assay

** HPLC= High performance liquid chromatography.

Table 2. Concentrations of oxytetracycline residues (ug/ g) in examined muscle and liver samples of rabbits. (n = 20).

| Sample | Maximum Permissible limit* | Samples above permissible limit | | Min. | Max. | Mean ± S.E |
|---|---|---|---|---|---|---|
| | | No. | % | | | |
| Muscle | 0.25 | - | - | 0.03 | 0.18 | 0.11±0.01+ |
| Liver | 0.25 | 4 | 20 | 0.07 | 1.15 | 0.67±0.04 |

+ = significant differences ($p<0.05$).
*Council Regulation (1990)

| Sample | Maximum Permissible limit* | Samples above permissible limit | | Min | Max | Mean ± S.E |
|---|---|---|---|---|---|---|
| | | No. | % | | | |
| Muscle | 0.05 | 1 | 5 | 0.02 | 0.07 | 0.05± 0.01+ |
| Liver | 0.05 | 3 | 15 | 0.04 | 1.15 | 0.34±0.02 |

Table 3. Concentration of ampicillin residues (ug/ g) in the exmined muscle and liver samples of rabbits (n = 20 ).

+ = significant differences ($p<0.05$)
*Council Regulation (1990)

Table 4. Concentrations of sulfadimidine residues (ug/ g) in the examined muscle and liver samples of rabbits. (n = 20).

| Sample | Maximum Permissible limit* | Samples above permissible limit | | Min. | Max. | Mean ± S.E |
|---|---|---|---|---|---|---|
| | | No. | % | | | |
| Muscle | 0.1 | 5 | 25 | 0.4 | 2.59 | 0.82± 0.13+ |
| Liver | 0.1 | 11 | 55 | 0.09 | 7.62 | 1.77± 0.45 |

+ = significant differences ($p<0.05$).
* Council Regulation (1990)

## DISCUSION

Extensive use of antibiotics caused some concerns about the development and distribution of antibiotic resistance in animal pathogenic microorganisms and consequently possible resistance in human pathogen strains. Drug residues left in edible tissue and environmental contamination, which may threat public health is another aspects of this tragedy. Use of antibiotics in animal feed is limited to special conditions in many countries, and growth promoter antibiotics (GPA) are prohibited in some countries *(10).*

The obtained results achieved in Table 1 indicated that only one sample of muscle of rabbits was positive for the presence of oxytetracycline residues by using FPT with an incidence of 5%, but the incidence of the same antibiotic in liver samples were 20%. Ampicillin residues were failed to be detected in all muscle samples, but detected in 15% of examined liver samples. Sulfadimidine residues were detected in 25% of examined muscle samples while for liver samples were 45%.

Regarding the quantitative detection of antibiotic residues in muscle and liver samples using of HPLC technique, incidence of both oxytetracycline and ampicillin residues were 10% in examined muscle samples. While incidence of oxytetracycline and ampicillin in examined liver samples was 30% 20%, respectively. The examined muscle and liver samples contained sulfadimidine residues in 40% and 65%, respectively. The differences between the examined samples of muscle and liver were significant ($p<0.05$) as a result of their concentrations of oxytetracycline.

Table 2 revealed that all examined of muscle samples were within maximum permissible limits of ***Council Regulation (EEC) (11)*** for oxytetracycline residues with a mean value of $0.11 \pm 0.0$ 1µ/g. The differences between the examined samples of muscle and liver were significant ($p<0.05$) as a result of their concentrations of oxytetracyclene.

Improper dosages of tetracyclines especially subtherapeutic doses may lead to the emergence of resistant bacteria. The organisms may become resistant to tetracyclines and to other agents *(12).*

Oxytetracycline is a broad-spectrum antibiotic used to treat a variety of infections and is used as a growth promoter in animals. In human being, about 60% of an ingested dose of oxytetracycline was absorbed from the gastrointestinal tract and then widely distributed in the body, particularly liver, kidney, bones and teeth *(13)* .However, the use of this compound may result in residues in animal meat especially if proper withdrawal times for treated animals have not been used *(14)*

Table 3 showed that the mean values of ampicillin residues in examined samples of muscle and liver was $0.05\pm 0.01$ and $0.34 \pm 0.02$, respectively, moreover , 5% of muscle and 15% of liver samples exceeded the safe permissible limits(0.05) for ampicillin residues. Our results of antibiotic residues in examined tissues in rabbits are nearly similar with those reported in previous studies *(15,16).*

The EU countries have established the values of maximum residual limits(MRLs) for ampicillin (AMP) antibiotic is set at 50 µg/kg , in all edible animal tissues (muscles, fat, liver, kidneys) ***Council Regulation (EEC) (11).***

The extensive use of penicillin may cause the presence of their residues in food products of animal origin and may have side effects to consumers. Moreover, penicillin residues in food products may be responsible for allergic reactions in human and promote the occurrence of antibiotic resistant bacteria *(17).*

Concerning to sulfadimidine residues, Table 4 recorded that it was 25% and 55% of exmined muscle and liver samples respectively, which higher than safe or maximum limits (0.1).The average of sulfadimidine concentration was $0.82\pm0.13$ and $1.77\pm0.45$ for muscle and liver samples, respectively.

Differences associated with sulfadimidine residue concentrations in the muscles and liver samples were significant ($p<0.05$).

The high incidence of sulfadimidine residue in muscle and liver samples may be due to extensive use of this drug in treatment of coccidiosis in rabbits by farmers or owners who could treat their animals and the miss use over dose and failure to observe the withdrawal periods can be common. At present, sulfonamides are seldom used for preventive purpose due to the development of new wide spectrum antibiotics, as well as due to an increasing resistance of the causative agents to them. However sulfonamides are still effective drug in the elimination of coccidiosis *(2).*

Sulphonamide residues present a potential risk to human health. Direct toxic or allergic reactions after administration of therapeutic doses of sulphonamides to humans have been described *(18).* Sulphonamides are known also for their negative effects on the thyroid gland in relation to the development of thyroid gland tumours *(19,20).*

The high incidence of antibiotic residues in examined liver samples may be attributed to that liver is responsible for metabolism and detoxication of the drugs by its microsomal enzymes *( 21),*also the failure to observe the withdrawal period for each antibiotic in rabbits before they were slaughtered for human consumption may also have been responsible for the residue accumulation in its tissue such as muscles and liver. Withdrawal times of antibiotics is considered as a safe way for avoiding its residues in human food of animal and poultry origin *(22,23).* Since the use of veterinary drugs especially antibiotic and sulfonamides is not adequately monitored and the prescription of antibiotics can easily be purchased so the use of wrong dose and mode of administration may be the cause of residue accumulation.

In Egypt there is no programs for monitoring drug residues in food especially antibiotics and sulfonamides and consequently, there are no available data on these residues in meat of rabbits and its organs. Moreover, there is no effective authority responsible for use of antibiotics for animal treatment.

Our results indicated that oxytetracycline, ampicillin and sulfadimidine residues tend to be higher in liver than in muscle samples. Most of the examined muscle samples do not have violative residues, but there are occasional samples that contain excessive amounts of these drugs. Usually these are a result of failure in observing the withdrawal period or from off-label use of an antibiotics and sulfonamides.

The authors recommended that the use of antibiotics in food producing animals' must be under control and should be given at recommended dose with appropriate supervision and adequate holding period or withdrawal period should be observed in all slaughtered animals including poultry and rabbits, following therapeutic use of antibiotics in treating sick animals, in addition to continuous training veterinarians to know the importance of withdrawal period in avoiding tissue residues.

## REFERENCES

*1.Schenck F.J. and Callery P.S. (1998):* Chromatographic methods of analysis of antibiotics in milk", J. Chromatog. A 812, 99-109.

*2.Kožárová I., Máté D.and Cabadaj R(2001):* Veterinary drug residues and the safety of foods of animal origin. Folia Vet . 45, 214-218.

*3.Salem, D.A. (1998):* Estamination of antibiotics, sulphonamides and nitrofurans residues in chicken meat. Assiut. Vet. Med. J. 39, 192-200.

*4.Gracey, J.F. and Collins, D.S. (1992):* Meat Hygiene, 9thEd, Bailliere Tidal, Oval Road, London.

*5.Doyle, M.E. (2006):* Veterinary Drug Residues in Processed Meats — Potential Health Risk *A Review of the Scientific Literature.* ©University of Wisconsin–Madison FRI BRIEFINGS.

*6.Bogaerts, R. and Wolf, F. (1980):* A standardized method for the detection of residues of antimicrobial substances in fresh meat. Flieschwirtschaft, 60: 672-673.

*7.Pieckova,E. and Van Peteghem,C.(2001):* Integrated strategy for the qualitative and quantitative analysis of residues of antimicrobial substances in food products. Food Research 40(4):275-283.

*8.Oka, H. ;Ito, Y.; Goto, T. ; Minami,T.; Yamamoto ,I.;Mastumoto,H.; Mizuno,Y; Tsuruta, M. and Hayashi,I.(2003):*Survey of residual tetracycle antibiotics and sulfa drugs in kidneys of diseased animalsin the Aichi prefecture, Japan. J. AOAC International, 86(3):494-500.

*9.Rosner,S.(2002):* Fundamental book of biostatistics 2nd Ed.,Publishing Co., New York, USA.

*10.Rahimi,SH., Karimi,T. M.A., Poorhassan, H. and Moghaddam, Z.(2005):* Drug-free chicken meat production. XVII th European Symposium on the Quality of Poultry Meat Doorwerth, The Netherlands, 23-26 May.

*11.Council Regulation (EEC) No2377 (1990):* Laying down a Cmmunity procedure for the establishment of maximum residue limits of veterinary medicinal products in food stuffs of animal origin. Official Journal L 224, 18(8): 1-8.

*12.Wilson, J. T., Golab, H. and Wright, L. (1982):* Residues tetracycline in meat products. Annals of Biochemistry, 10, 40-45.

*13.Czeizel A.E., Rockenbauer, M. and Olsen J., (1998).* Use of antibiotics during pregnancy". European J. of Obstetrics and Gynecology and Reproductive Biology, 81 1-8 \ 6.

*14.Long A.R., Hsieh L.C, Malbrough M.S, Short, C.R. and Barker ,S.A.(1990):* Matrix Solid-Phase Dispersion (MSPD) Isolation and Liquid Chromatographic Determination of Oxytetracycline and Tetracycline and Chlorotetracycline in Milk."J. Assoc Anal. Chem. 73(3), pp.379-84.

*15.Daoud,G.R.,(1991):*Studies on the withdrawaltime of some antibiotics from broilers and rabbits .M. V. Sc. Thesis. Fac. Vet. Med., Moshtohor, Zagazig University.

*16.Basyoni,S.R.(2003):* Detection of some antibiotic residues in slaughtered quails and rabbits.7th National Conference on Environmental studies andResearch.,20-23 April, 2003.J. Environmental Science (Special issue),Vol.6(3):757-7672

*17.Kowalski, P. and Lucyna,K. (2007):* Determination of penicillin antibiotic in poultry muscle by capillary electrophoresis. Bull Vet Inst Pulawy 51, 595-598.

*18.Booth, N.H.(1988):* Toxicology of drug and chemical residues in eddible tissues of animals. In: *Veterinary Pharmacology and Therapeutics*, edited by H.R. Adams (Ames: Iowa State University) ,pp.1149-120

*19.Swarm R.L, Roberts G.K.S., Levy A.C., Hines L.R.(1973):* Observation in the thyroid gland in rats following the administration of sulphamethoxazole and trimethoprim. Toxicol Appl Pharmacol, 24, 351-363.

*20.Ahmed M.S.and Ahmed N. (1989):* Drug residues in poultry meat and eggs – public health importance. Poultry Adviser., 22, 53-58.

*21.Arichimbault,P., Boutier,C.and Muscal,G. (1978):* Ampicillin residues in poultry after oral administration to laying hens. Revue. Med. Vet., 129(11)137-144.

*22.Anadon,A., Martinez- Lannange, M.R., Diaz,M.J.Velez,C.and Bringas, P.(1990):* Pharmacokinetic and residue studies of quinolone compounds and alaquinodox in poultry.Ann.Res.Med.Vet.,129(11):137-145.

*23.Mignot,A.Lefebre,M.A.and Millerioux.L. (1993):* Determination of spiramycin and neomycin in biological samples (plasma, muscle, liver, kidney, skin with with fat ) from a residue study of chicken.CEPHAC Report NO.C.D 508, june.

## الملخص العربى

### تقدير متبقيات بعض المضادات الحيوية والسلفا فى لحوم وأكباد الأرانب

سهير رشاد بسيونى ، عبد العزيز أحمد حلمى بر *
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أجريت هذه الدراسة لمعرفة مدى وجود كل من الأوكسيتتراسيكلين و الأمبيسيلين والسلفاديميدين فى عينات عضلات واكباد الأرانب المذبوحة فى محلات الدواجن والأرانب بمدينة طنطا بمحافظة الغربية كيفا باستخدام الطريقة الميكروبيولوجية Four plate test (FPT) للتقدير الكيفى وكذلك تم استخدام جهاز الكروماتوجرافى السائل العالي الجودة ( HPLC) للتقدير الكمى فى العينات. واسفرت النتائج عن أن العينات كانت ايجابية لوجود متبقيات بعض هذه الأدويه بنسب مئويه مختلفة وكان متوسط تركيزات بقايا الأوكسيتتراسيكلين و الأمبيسيلين والسلفاديميدين فى عينات عضلات الأرانب هو ٠،١١ ± ٠،٠١و٠،٠٥±٠،٠١و٠،٨٢ ±٠،١٣ على الترتيب أما فى عينات الكبد فكا ن متوسط تركيزات بقايا كل من الأوكسيتتراسيكلين و الأمبيسيلين والسلفاديميدين هو ١،٧٧±٠،٤٥و ٠،٦٧±٠،٠٤و ٠،٣٤ ± ٠،٠٢على الترتيب.كما أظهرت النتائج أن٥% و١٥% من عينات عضلات الأرانب و اكبادها أعلى من الحدود المسموح بها للأمبسيلين وان ٢٥% من اكباد الأرانب أعلى من الحدود المسموح بها للأوكسيتيترسيكلين , أما بالنسبة للسلفاديميدين فان ٢٥% و ٥٥% من كل من عينات العضلا ت والأكباد كانت أعلى من الحدود المسموح بها. هذا وقد تم مناقشة الأهمية الصحية لهذه البقايا وخطورتها على صحة المستهلك , وكذلك تم وضع التوصيات اللازمة لتجنب وجود هذه البقايا فى لحوم وأكباد الأرانب.