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Prevalence of Antimicrobial Residues in Eggs, Tissue and Feed Samples in the State of Kuwait

انتشار متبقيات المضادات الحيوية في عينات البيض واللحوم والأعلاف في دولة الكويت

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ABSTRACT: A total of 238 locally produced and imported eggs, tissue (meat, poultry and aquacultured fish) and feed and feedstuffs samples were collected at different seasonal periods from different farms and retail outlets in Kuwait and screened for the presence of β-lactams, tetracyclines, sulfonamides, streptomycin, macrolides and chloramphenicol (799 tests) using Charm II system. The results indicated that all of the 222 tests performed on table egg samples were negative for the analyzed antimicrobial residues indicating adherence to the guidelines for antimicrobial use and withdrawal. Similarly, all of the 268 tests performed on tissue samples were negative for the analyzed antimicrobial residues except for chloramphenicol. These chloramphenicol positive samples (10.8%) were for chicken parts imported from China. For feed and feedstuff samples, all of the 66 tests performed were negative for β-lactams residues. Out of the 79 feed and feedstuff samples analyzed for tetracyclines residues, 4 broiler diet and concentrate samples (5%) were above the tetracyclines MRL (100 ppb). On the other hand, results have revealed a widespread of sulfonamide residues and to a less extent chloramphenicol in tested feed and feedstuff samples. The Charm II system was reliable for rapid screening of antimicrobial residues. In general, results obtained in our study necessitate more effective and well planned national antimicrobial residues surveillance programs focusing particularly on samples imported from highly risk sources.

Keywords: *Antimicrobial Residues, Eggs, Meat and Meat Products, Poultry, Aquacultured Fish, Charm II System, Kuwait.*

المستخلص: تم تقييم بقايا مجموعات المضادات الحيوية (البيتا لاكتام، والتتراسايكلين، والسالفوناميد، والكلورامفينيكول، والستربيتومايسين، والمايكرولايد)، في دولة الكويت، باستخدام جهاز نظام الشارم الثاني، في 238 عينة (779 اختبار) تشمل كل من البيض، واللحوم، والأسماك، والأعلاف بمكوناته الأولية المنتجة محليا أو المستوردة. وقد تم تجميع العينات في فترات موسمية متباينة من مزارع مختلفة وأماكن بيع بالتجزئة. وأشارت نتائج عدد 222 اختبار لعينات البيض خلوها من بقايا

مجموعات المضادات الحيوية مما يعكس التزام مزارع إنتاج البيض بتعليمات استخدام المضادات الحيوية وفترات المنع. وبنفس الطريقة، دلت نتائج عدد 268 اختبار أجريت على عينات من اللحوم والأسماك خلوها من بقايا مجموعات المضادات الحيوية ما عدا الدجاج المستورد من الصين فقد كانت نسبة العينات الايجابية فيه (أعلى من المسوح به دوليا) هي 10.8 % لبقايا المضاد الحيوي الكلورامفينيكول. و قد دلت نتائج تحليل عينات العلف، ومكوناته الأولية، إلى خلوها من بقايا البيتا لاكتام، في حين كانت نسبة العينات الإيجابية لبقايا التتراسايكلين والكلورامفينيكول والسالفوناميد هي 5.1 % و 8.5 % و 70.2 % على التوالي، مما يشير إلى انتشار واسع لبقايا هذه المجموعات من المضادات الحيوية في الأعلاف ومكوناته الأساسية. وتشير تحاليل مطابقة النتائج إلى أن نظام شارم الثاني المستخدم في الدراسة يمكن أن يعول عليه للكشف السريع عن بقايا مجموعات المضادات الحيوية في الأغذية والأعلاف. وبشكل عام، يتضح من الدراسة الحاجة إلى وضع برامج مسح دورية وطنية على بقايا مجموعات المضادات الحيوية في الأغذية والأعلاف وبشكل خاص في العينات المستوردة من المصادر ذات الخطورة العالية.

كلمات مدخلية : *متبقيات المضادات الحيوية، البيض، اللحوم ومنتجاته، الدواجن، الأسماك المستزرعة، أعلاف، جهاز نظام الشارم الثاني، دولة الكويت.*

INTRODUCTION

Antimicrobial agents are a chemically heterogeneous group of small organic molecules of microbial origin that, at low concentrations, are deleterious to the growth or metabolic activities of other microorganisms (Thomashow and Weller, 1996). Over 50% of antimicrobial agents produced by pharmaceutical companies worldwide are used in animal agriculture. It is estimated that more than 2000 antimicrobial agents are currently licensed for use as veterinary medicines. The drugs are used not only to treat infections in sick and injured animals, but also to prevent infections (prophylaxis use) and as growth-promoting additives in feed. In the latter two cases, the antimicrobial agents are used at concentrations lower than those used for treatment; a potentially dangerous practice since it can encourage the production of antibiotic resistant strains of bacteria that are pathogenic to animals and humans (Khachatourians, 1998; Simonsen, *et al.* 1998). The use of sub-therapeutic levels of antibiotics as growth promoters, or for prophylaxis use, remains a concern and developed countries are phasing out their use as feed additives. Moreover, antibiotic residues in food products of animal origin are one of the sources of concern among the public and medical health professionals. These residues may potentially cause allergic reactions and alter the dynamics of the microflora in the intestinal tracts of humans (Witte, 1998).

Acceptable levels of antimicrobial residues in eggs, tissue (meat, poultry and fish) and feed and feedstuffs samples have been set by expert bodies in many countries, regions and agencies including the European Union, the United States, Canada, Australia and Codex Alimentarius Commission, for all drugs used in agriculture. The levels are expressed as tolerance or safe levels (T/SL) or maximum residue levels (MRL) and range according to the class of antibiotic from 4 to 100 parts per billion (ppb) levels below which it is considered that the drug may be safely used without harming the consumer. However, levels above the T/SL or MRL are by inference not safe, reflect misuse of the drugs, and should not be exposed to the consumer (Van Eeckhout, *et al.* 1998).

Antibiotic residues in eggs, tissue and feed and feedstuffs are measured by a variety of techniques which differ in principle, detection limits, specificity, need for sample treatment, speed, throughput and cost per sample (Kanfer, *et al.* 1998; Schugerl and Seidel, 1998; Marzo and Bo, 1998; Niessen, 1998). Some of these methods are considered to be screening procedures, and others are definitive or confirmatory methods with greater accuracy. Generally, the methods are chemical, biological, microbiological or immunological. Chemical, biological, and immunological methods measure residue concentrations in mass, and microbiological methods measure effect with semiquantitative results.

The Charm II system is considered to be a screening procedure for the presence of antimicrobial residues in food products of animal origin. The principle of the Charm II system for class drug residue analysis is based on the use of two reagents: a radio-labeled [^{14}C] or

[^{3}H] antimicrobial drug (tracer) and a binding reagent (microbial or immunochemical receptor). When the receptor is added to milk containing antimicrobial residue, the contaminating residue binds to the receptor. When the tracer is added, any antimicrobial residue in the sample bound to the receptor will prevent the tracer from binding to these sites. The bound receptor/tracer complex is then separated from the unbound tracer by centrifugation and measured with a liquid scintillation counter (counts per minute). Count reading of sample is then compared either to counts of a negative or a positive standard sample.

The most effective way to reduce the rate of evolvement of drug resistance and to prevent toxic residues from appearing in consumed foods of animal origin is to control the use of antimicrobial agents. Control of the use of antimicrobial agents and prevention of their misuse relies primarily on an effective, well-planned and conducted, national drug residue surveillance program(s). In the State of Kuwait, at the present time, information on the levels of antimicrobial residues in locally produced and imported eggs, tissue and feed and feedstuffs samples remains unknown. No surveys have been undertaken to assess residue concentrations or seasonal variations and the decision on the safe use of antimicrobial agents in animal production is left to the farming community.

Due to lack of data on the presence of antimicrobial residues in locally produced and imported eggs, tissue and feed and feedstuffs samples, the outcome of this study will provide valuable baseline information for local governmental authorities for effective monitoring for the use and misuse of agricultural antimicrobial agents. Such data will also be important for other Gulf Cooperation Council (GCC) Countries, since such countries share similar farming practices and import comparable foodstuffs.

MATERIAL AND METHODS

Sampling

The snapshot sampling protocol plan used in this study is presented in Table 1. The objective of the snapshot survey was to collect a total of 238 eggs, tissue (meat, poultry and fish) and feed and feedstuffs samples from as many outlets and farms as practically possible in a short time period as possible. The snapshot sample collection was repeated during the period from April 2004 to February 2005 to ensure that the time of production and seasonal variation have been taken into consideration, and therefore, comparison will be valid, and any trends in the use of antimicrobials on a state scale can be determined.

A minimum of three tissue and feed and feedstuffs samples of selected brands were collected from outlets according to the sampling plan (Table 1). Of the three collected samples, one sample was used for the screening test, while the remaining two samples were used for further confirmation tests, if necessary. In this study, only fresh table eggs were used to detect antimicrobial drug residues. Ten egg samples of selected brands were randomly picked, cracked open into a clean container and whipped with a spatula until the color was uniform.

Sample and Standard Assay Procedure

The Charm II 7600 system (Charm Sciences Inc., USA) was used to screen for the presence of β-lactams, sulfonamides, chloramphenicol, streptomycin and macrolides in egg samples and for the presence of β-lactams, tetracyclines, sulfonamides, and chloramphenicol in tissue (meat, poultry and fish) and feed and feedstuffs samples. The used Charm II kits were sensitive to detect antimicrobial residues at the EU maximum residue limits (Council Regulation 2377/90/EEC and amendments). The test kits comprised of tablet reagents for an antimicrobial class, positive control standards, negative control standards and buffer solutions along with scintillation liquid. Sample and standard preparation as well as Charm II test procedure for the residues of six antimicrobial classes was performed following the protocols given in the Charm Operator Manuals received with each test kit. Control points for each class of antimicrobial residues analyzed as well as for each new lot of reagents and kits were determined. The control point is a measurement (counts per minute) of the radio-labeled drug (tracer) binding to the specific binding sites (receptor) using a liquid scintillation counter and determined from a negative control or positive spiked sample.

Table 1. Collection Sampling Plan for Locally Produced and Imported Eggs, Tissue, Feed and Feedstuuf Samples in the State of Kuwait During the Year 2004/05.

Food Item	Testing Frequency	Sampling Plan		Total Samples
		Summer	Winter	
Eggs				
Imported	4	2 (5 brands)	2 (5 brands)	20
Local	4	2 (10 brands)	2 (10 brands)	40
Sub-Total				**60**
Local Meat and Meat Products				
Whole Chicken	4	2 (5 brands)	2 (5 brands)	20
Sheep Meat	4	2 (2 brands)	2 (2 brands)	8
Sub-Total				**28**
Imported Meat and Meat Products				
Chicken Parts	4	2 (5 brands)	2 (5 brands)	20
Whole Chicken	2	1 (5 brands)	1 (5 brands)	10
Sheep Meat	2	1 (3 brands)	1 (3 brands)	6
Beef Meat	2	1 (6 brands)	1 (6 brands)	12
Sub-Total				**48**
Fish and Aquacultured Fish				
Imported	5	2 (2 brands)	3 (2 brands)	10
Local	2	1 (4 farms)	1 (4 farms)	8
Sub-Total				**18**
Imported Mixed Feed and Feedstuffs				
Animal Feed	10	5 (2 kinds)	5 (2 kinds)	20
Poultry Feed	8	4 (4 kinds)	4 (4 kinds)	32
Feedstuffs	8	4 (4 kinds)	4 (4 kinds)	32
Sub-Total				**84**
Total				**238**

Confirmation of Charm II system Results with LC/MS/MS and GC/MS/NCI

Selected samples analyzed by the Charm II system for tetracycline (2 samples), chloramphenicol (4 samples) and sulfonamide (5 samples) residues were confirmed using gas chromatography mass spectrometry in negative chemical ionization mode (GC-MS-NCI) and liquid chromatography-mass spectrometry (LC/MS/MS) in a private laboratory (Maxxam Analytics Inc., Canada). Samples intended to be analyzed for tetracycline and chloramphenicol residues were carried out by LC/MS/MS with a method detection limit (MDL) of 100 ppb for tetracycline and oxytetracycline and 1 ppb for chloramphenicol, respectively. Sulfonamide residues were analyzed by GC/MS/NCI with a MDL of 0.02 ppb for sulfadiazine, sulfadimethoxine, sulfamethazine, sulfaquinoxaline and sulfathiazole. All confirmation tests were made in duplicates.

RESULTS AND DISCUSSION

Antimicrobial Residues in Eggs

Fresh imported and local table egg samples were collected from outlets during the period from May 2004 to July 2005. Imported eggs were from India, Lebanon, Saudi Arabia, Turkey and the Netherlands. The collected local egg samples represent the products of the major 10 layer poultry farms in Kuwait including the Public Authority for Agriculture and Fisheries Resources (PAAFR) layer poultry farm. The sample size, 67% of the collected egg samples were locally produced and 33% were imported, was chosen to reflect the consumption pattern of eggs in Kuwait. Samples were analyzed for the presence of β-lactams, sulfonamides, chloramphenicol, streptomycin and macrolides.

All of the 71 and 151 testes performed on imported and local table egg samples, respectively, were negative for the analyzed antimicrobial residues. Similar results were obtained in Barbados for streptomycin, macrolides, sulfonamides, and tetracyclines residues in eggs using Charm II system (Hall, *et al.* 2003). In their study, a total of 195 tests were performed on eggs during the interval between1996 and 2003. Of these, only six yielded a positive result namely for tetracyclines. On the other hand, prevalence of antimicrobial residues in eggs in Trinidad using Charm II system revealed that out of 184 eggs tested, 12 (6.5%) were contaminated with sulfonamide compared with 7 (3.8%) and 5 (2.7%) were contaminated with macrolides and tetracycline, respectively (Adesiyun, *et al.* 2005). Therefore, it was concluded that the presence of antimicrobial residues in table eggs could be of public health significant to consumers in Trinidad. The absence of antimicrobial residues in all table egg samples tested in our study can be interrelated to several reasons including: 1) good hygienic and production practices in the layer houses resulting in less infection; 2) localized use of antimicrobial drug for the treatment of infected layer and 3) discriminated use of antimicrobial agent and the right implementation of recommended withdrawal time by large poultry industry. However, it is recommended to routinely monitor the hygienic and production practices at layer farms as well as the inspection of table eggs for antimicrobial residues prior to marketing.

Antimicrobial Residues in Tissue Samples

Imported and local meat and meat products and aquacultured fish samples were collected from outlets in Kuwait during the period from July 2004 to July 2005. Imported chicken parts samples originated from China and Brazil, while whole chicken samples were from Brazil and Saudi Arabia. All imported chicken samples (parts and whole) were frozen. Local whole chicken samples represent the products of the five major broiler farms in Kuwait. Approximately, 50% of the collected local whole chicken samples were fresh meat and the remaining were frozen.

Imported sheep meat samples were from Australia, Iran and China, while beef meat samples were from China, India and Brazil. Half of the collected imported sheep and beef samples were fresh meat while the remaining samples were frozen. All collected local sheep meat samples were fresh meat. Imported aquacultured Bulti (Tilapia nilotica) and Subaiti (silver black porgy) were from Egypt and UAE, respectively, while local Bulti samples were from 4 aquaculture farms in the Wafra agriculture area (southern part of Kuwait).

The sample size, presented in Table 2, was selected based on personal communication with managers' of various cooperative societies in Kuwait to reflect the consumption pattern for local and imported fresh and frozen meat and meat products and aquacultured fish samples. The collected tissue samples were screened for β-lactams, tetracyclines, sulfonamides and chloramphenicol.

All of the 148 tests performed on imported meat and meat product samples were negative for the analyzed antimicrobial residues, except for chloramphenicol (Table 2). Out of 37 samples analyzed for chloramphenicol, 4 samples (10.8%) were contaminated with chloramphenicol. These positive samples were for frozen chicken parts imported from China. Similarly, all of the 76 and 44 tests performed on local meat and meat products and on imported and local aquaculture fish samples were negative for the analyzed antimicrobial residues. Al-Ghamdi, *et al.* (2000)

Table 2. Antimicrobial Residue Calsses Analyzed in Imported Meat and Meat Products Samples Collected During the Year 04/05.

Commodities	β- lactams			Tetracycline			Sulfonamides			Chloramphenicol		
	Samples Analyzed	Samples Below MRL	Samples Above MRL	Samples Analyzed	Samples Below MRL	Samples Above MRL	Samples Analyzed	Samples Below MRL	Samples Above MRL	Samples Analyzed	Negative Samples	Positive Samples
Chicken parts	15	15	0	15	15	0	15	15	0	15	11	4
Whole chicken	8	8	0	8	8	0	8	8	0	8	8	0
Sheep meat	6	6	0	6	6	0	6	6	0	6	6	0
Beef meat	8	8	0	8	8	0	8	8	0	8	8	0
Total	**37**	**37**	**0**	**37**	**37**	**0**	**37**	**37**	**0**	**37**	**33**	**4**

have shown that out of 33 chicken samples from the eastern province of Saudi Arabia, 23 (69.7%) were contaminated with tetracycline residues indicating widespread misuse of tetracycline agents and lack of implantation of recommended withdrawal times. Although, our study indicates that local tissue samples (meat, poultry and fish) at the time of the survey have no incidence of antimicrobial residues, yet more frequent testing should be directed to tissue samples imported from highly risk sources.

Antimicrobial Residues in Imported Feed and Feedstuffs

Antimicrobial residue classes detected in imported feed and feedstuff samples are shown in Table 3. Samples were collected from retail outlets in Kuwait and from PAAFR feed control laboratory during the period from February 2004 to July 2005. The collected cattle and sheep feed were imported from Australia, Egypt, and Iran, while poultry feed and concentrates were imported from India, Egypt, Iran and EU countries. Feedstuff constituents include corn, barley, millets and soybean meal were imported from India, Iran and UAE. Antimicrobial classes analyzed in imported feed and feedstuffs samples were β-lactams, tetracyclines, sulfonamides and chloramphenicol.

All of the 66 feed and feedstuff samples tested negative for β-lactams residues. Similarly, all of the 79 feed and feedstuff samples tested negative for tetracyclines residues except for 4 samples (5%) that were above the tetracyclines MRL (100 ppb). These presumptive positive samples were for broiler diets and concentrates. On the other hand, results have revealed a widespread of sulfonamide residues and to a lesser extent chloramphenicol in tested feed and feedstuff samples. For sulfonamide residues, 35% of tested animal feed, 100% of tested poultry feed and 64.5% of tested feedstuffs were above the MRL (100 ppb) whereas 17.6% of tested animal feed, 4.3% of tested poultry feed and 6.6% of tested feedstuffs were positive for chloramphenicol (MRL cannot be established).

In general, the results shown in Table 3 illustrates that sulfonamides was the most frequently found antimicrobial residues in feed and feedstuff samples, followed by chloramphenicol, and tetracyclines. No samples were found positive for β-lactams residues. Internationally, antimicrobial agents are used largely as growth promoters to improve animal's feed utilization and production and this constitute a large proportion of its usage. For example, in Australia, statistics for the years 1992 to 1997 showed that 55.8% of imported antimicrobial agents were for use in stock feed, 36.4% for human and 7.8% for veterinary (Joint Expert Technical Advisory Committee on Antibiotics Resistance, 1999).

Recently, attention has started to focus on antimicrobials in animal feed. Much of the concern has been directed to the potential spread of antimicrobial residues and antimicrobial resistant bacteria from treated animal via the food chain. Therefore, the continued use of antimicrobial growth promoters continue to be a question in reports from committees in many countries (Barton, 2000).

Table 3. Antimicrobial Residue Calsses Analyzed in Imported Mixed Feed and Feedstuffs Samples Collected During the Year 04/05.

Commodities	β- lactams			Tetracycline			Sulfonamides			Chloramphenicol		
	Samples Analyzed	Samples Below MRL	Samples Above MRL	Samples Analyzed	Samples Below MRL	Samples Above MRL	Samples Analyzed	Samples Below MRL	Samples Above MRL	Samples Analyzed	Negative Samples	Positive Samples
Animal Feed												
Cattle Feed	11	11	0	13	13	0	12	9	3	10	10	0
Sheep Feed	7	7	0	5	5	0	5	2	3	7	4	3
Total	**18**	**18**	**0**	**18**	**18**	**0**	**17**	**11**	**6**	**17**	**14**	**3**
Poultry Feed												
Broiler Diets	4	4	0	8	6	2	7	0	7	4	4	0
Layer Diets	6	6	0	6	6	0	6	0	6	6	6	0
Broiler Concentrates	6	6	0	6	4	2	6	0	6	4	3	1
Layer Concentrates	5	5	0	9	9	0	7	0	7	9	9	0
Total	**21**	**21**	**0**	**29**	**25**	**4**	**26**	**0**	**26**	**23**	**22**	**1**
Feedstuffs												
Corn	3	3	0	3	3	0	3	2	1	3	3	0
Barley	4	4	0	4	4	0	4	4	0	4	4	0
Millets	4	4	0	4	4	0	4	4	0	4	2	2
Soybean meal	16	16	0	21	21	0	20	1	19	19	19	0
Total	**27**	**27**	**0**	**32**	**32**	**0**	**31**	**11**	**20**	**30**	**28**	**2**

Confirmation of Charm II System Results with LC/MS/MS and GC/MS/NCI

For tetracyclines, one presumptive negative (egg sample) and 1 presumptive positive sample (feed sample) as determined by the Charm II system were confirmed by LC/MS/MS. Results indicated that presumptive negative sample was also found negative by LC/MS/MS and presumptive positive sample was found to contain tetracycline at less than the MDL of 100 ppb and oxytetracycline at 6900 ppb.

For chloramphenicol, 2 presumptive negative (1 egg and 1 feed sample) and 2 presumptive positive feed samples as determined by the Charm II system were confirmed by LC/MS/MS. Results indicated that the two presumptive negative samples were also negative by LC/MS/MS and traces of chloramphenicol were found in the presumptive sample at less than the MDL of 1 ppb.

For sulfonamides, 3 presumptive negative (2 feed and 1 egg samples) and 2 presumptive positive feed samples as determined by the Charm II system were confirmed by GC/MS/NCI for sulfadiazine, sulfadimethoxine, sulfamethazine, sulfaquinoxaline and sulfathiazole with MDL of 0.02 ppb. Results indicated that presumptive negative sample was also found negative by GC/MS/NCI and presumptive positive samples were found to contain sulfathiazole at 0.15 ppb for one sample and 0.12 ppb for the other presumptive positive sample.

The confirmation results indicate that the Charm II system was reliable for rapid screening of antimicrobial residues and the few cases of presumptive positive sample obtained by Charm II system and found at less than the MDL of the GC-MS-NCI and LC/MS/MS necessitates the need to conduct a confirmatory analysis with conventional methods. Overall, the rapid screening ability of the Charm II system was essential to rule out negative results and reduce the cost of analyses using highly sophisticated and time consuming conventional methods.

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