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ARTICLE



# Antimicrobial residues survey by LC-MS in food-producing animals in Lebanon

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**ABSTRACT**

The treatment of animals with antimicrobial products may lead to the contamination of edible tissues by their residues, which may represent a risk to human health. Therefore, this study aimed to determine the level of antimicrobial residues in food-producing animals (chicken, beef, and milk) in Lebanon. A total of 310 samples were collected and analysed using an LC-MS /MS for the determination of 48 compounds belonging to different families in order to map their compliance according to the European Commission decision 2002/657/EC. Results show that 60% of the analysed samples were not contaminated by any residue, while 12% presented a concentration higher than the MRLs for tetracyclines, sulphonamides, quinolones, and macrolides. Results revealed that chicken were the most contaminated by antimicrobial residues, when compared to beef and milk. The obtained results demonstrate the uncontrolled use of antimicrobials in some Lebanese farms and claim for better management of livestock.

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**KEYWORDS**
Antimicrobials; milk; edible animals; LC/MS/MS screening

## Introduction

Antimicrobial veterinary products are widely administered in livestock, to not only treat or prevent infections, but also promote growth (Aarestrup 2012). Approximately 80% of all food-producing animals receive medication for part or most of their lives (WHO 2017). Their usage may leave residues in the edible tissues, which is considered an important source of potential toxicological risk to the consumer (Kabir et al. 2004; Jafari et al. 2007; Sappington et al. 2011). Moreover, these residues can lead to an allergic reaction, a disorder of intestinal flora or emergence and spread of drug-resistant bacteria. Therefore, the European Union (EU) has banned the nontherapeutic growth-promoting use of antimicrobials (European Commision 2002). In 2010, the European regulation No. 37/2010 on pharmacologically active substances has established the Maximum Residue Limits (MRLs) for edible biological matrices, such as milk and meat.

In Lebanon, a rapid growth of the livestock productivity has been shown that it led to enormous use of drugs. The population of 4.429 million in 2013 increased by 37% since 2011 according to the Lebanese government, therefore domestic demand for foodstuffs of animal origin has increased rapidly. According to the latest survey by the Ministry of Agriculture in 2009, the number of cattle, sheep, and goat was estimated to 74.9, 372.1, and 430.1 thousand, respectively. However, the quantity of food-producing animal is not sufficient, for this reason, Lebanon import meat which was estimated to 112.5 thousand tonnes coming from many suppliers of the European Union (Germany, Holland, and Ireland) and from South America (Brazil and Colombia).

To estimate the concentrations of the antimicrobial residues in animal food and to design strategies to minimise their exposure to these compounds, there is a need for reliable analytical methods to evaluate their values at trace levels ($\mu g.L^{-1}$). In addition, there is a lack of data concerning the presence of the antimicrobial residues in food animal produced in Lebanon. Several analytical methods for the determination of multiclass veterinary drugs were used including microbiological methods, enzyme immunoassays, and chromatographic analyses usually coupled with derivatisation steps (Samandoulougou et al. 2015; Rama et al. 2017; Aydin Unsal et al. 2018). Microbiological inhibitory plates are simple and inexpensive but these methods are in some cases not enough sensitive (false-negative results) and may provide a false positive result (Pikkemaat 2009). Rapid test kits are also employed but have a drawback to cover only a few targeted compounds or compounds belonging to the same family of

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antimicrobials (Schlemper and Sachet 2017). Liquid chromatography coupled to mass spectrometry (MS) using a quadrupole mass analyser (LC-MS/MS) has become the most appropriate method for the residue analysis with its high selectivity, sensitivity, decisiveness, and its applicability to determine the polar and/or non-volatile compounds without derivatisation, including both electrospray and atmospheric pressure chemical ionisation methods (Mokh et al. 2017; El Hawari et al. 2017). The majority of the residue analysis methods, use low-resolution mass spectrometry instruments in "multiple reaction monitoring" (MRM) or "selected reaction monitoring" (SRM) modes, allowing the detection of target analytes below the MRLs.

The present work was therefore designed to assess the level of antimicrobial drug residues in beef, chicken, and milk consumed in different Lebanese regions. The sampling strategy is aimed to detect illegal treatments and to control compliance with the MRLs outlined in the European legislation. However, this study searched to identify samples, which may contain non-compliant residues in food animal origin through screening methods by LC-MS/MS for different groups of antimicrobials, including sulphonamides, lincosamides, macrolides, tetracyclines, β-Lactams, and aminoglycosides. In this work, the method developed and optimised by Gaugain-Juhel et al. (2009) was used.

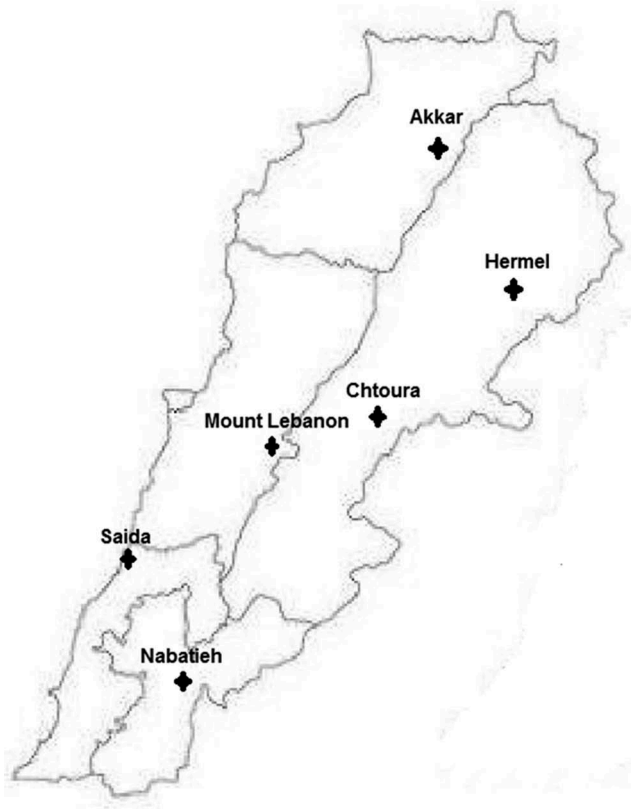


Figure 1. Overview of the sampling sites in Lebanon.

## Materials and methods

### Chemicals and reagents

Drug standards were supplied by Sigma-Aldrich (St. Louis, MO, USA), Dr Ehrenstorfer (Augsburg, Germany), TRC Toronto, and European pharmacopoeia. The purity of all products was higher than 90%, except for tylosin A (86.4%) and for erythromycin (89.4%). LC/MS grade acetonitrile (MeCN) and methanol (MeOH) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Trichloroacetic acid (TCA) and ammonium acetate from Merck (Darmstadt, Germany) then pentafluoropropionic acid (PFPA) from Sigma Aldrich (St. Louis, MO, USA) were analytical reagent grade. Deionised water with a resistivity of 18.2 mΩ was prepared with Barnstead-Easy pure II from Thermo Fisher scientific (Hudson, USA). Purified extracts were filtered through a 0.2 µm Ultrafree-CL Centrifugal filter with a low-binding Durapore PVDF membrane (Millipore, Molsheim, France).

### Sample collection

Three hundred ten samples of chicken (155), beef (56), and Milk (99) were collected from June to November in 2013 for antimicrobials residues analysis. The samples were purchased from different animal farms and markets in six different city areas as shown in Figure 1. They are representatives of commonly consumed commodities in Lebanon. Samples were placed in sterile bags in an ice chess box, labelled and then transported to the laboratory for processing. The homogenised samples were immediately stored at 4°C and extracted within 24 h.

### Solutions and standards

Individual standard of each antimicrobial (~10 mg) was weighed in a 25 mL polypropylene volumetric flask. Sulphonamides, macrolides, quinolones, and trimethoprim were dissolved in acetonitrile and tetracycline antibiotics were prepared in methanol whereas distilled water was used for the β-lactams and aminoglycosides. Working standard solutions (1–5 µg.mL$^{-1}$) were prepared in the mobile phase from the stock solutions for the tuning of the ESI source and for MS/MS transitions settings. From these stock solutions, suitable concentrations for spiking mixture were also dissolved in ultrapure water to be used during the identification process. A 5% TCA solution was obtained by dissolving 50 g of trichloroacetic acid in 1 L of water. A 2 M ammonium acetate solution was prepared by dissolving 15.4 g of ammonium acetate in 100 mL of water and then diluted

by a tenth to obtain a 0.2 M. Then, 0.1% PFPA solution was also prepared by adding 1 mL in 999 mL of water for the mobile phase.

### Sample extraction

Two different extractions were applied, but sulfaphenazole and demeclocycline served as an internal standard in both extractions. Samples were allowed to thaw followed by a quick homogenisation by shaking before taking up a test portion of 2 mL for milk and 2 g for chicken and beef which 200 µL of internal standard (1 mg/kg) and 800 µL of water were added.

### Extraction of β-Lactams, macrolides, and sulphonamides

In addition of 8 mL of acetonitrile, the test portion were stirred for 10 min and then centrifuged at 14000 g for 5 min. Six millilitres from the supernatant was evaporated under nitrogen flow, the residual volume was dissolved in 0.6 mL of 0.2 M ammonium acetate, and then filtered onto a 0.45 µm Millex HV filter of 13 mm diameter.

### Extraction of tetracyclines, quinolones, aminoglycosides, and lincosamides

In the addition of 8 mL of 5% TCA solution, the test portion was stirred for 10 min and then centrifuged at 14000 g for 5 min. About 1 mL of the supernatant was filtered onto a 0.45 µm Millex HV filter.

### LC-MS/MS analysis

Analysis was performed with an Agilent 1200 HPLC system equipped with a reversed-phase Eclipse XDB-$C_{18}$ column (4.6 mm × 150 mm × 5 µm), interfaced to an Agilent 6410 triple quadrupole mass spectrometer (Agilent Technologies, Waldbronn, Germany) via an electrospray ionisation source, operated in positive mode (ESI+). The MS/MS parameters were optimised as follows: capillary voltage, 4000 V; temperature, 350° C; nebuliser pressure, 40 psi and gas flow rate, 9 L. $min^{-1}$. The mobile phase consisted of water containing 0.1% (v/v) PFPA and acetonitrile and the gradient elution program is presented in Table 1. The acquisition time for analysis was fixed at 33 min, the column's temperature set at 30°C, and the injection volume of the extract was 25 µL. Optimisation of the cone voltage and collision energy for each product ion that gave the highest abundance was performed. Multiple reaction monitoring was used. Detailed parameters and method sensitivity are presented in Table 2. The limit of detection (LOD) and limit of quantification (LOQ) were calculated with the equations LOD = 3.3 sd/slope and LOQ = 10 sd/slope, where sd/slope is the standard deviation of the response at low concentrations, divided by the slope of the calibration curve. The values of LOD and LOQ ranged from 1 to 308 µg/kg. In order to eliminate false-positive results and ensure the system to be under control, internal quality control during sample analysis was applied by spiking blank matrices.

Table 1. Gradient elution program for the liquid chromatographic separation.

| Time (min) | Flow (ml/min) | Acetonitrile | Water 0.1%PFPA |
|---|---|---|---|
| 0 | 0.4 | 10 | 90 |
| 6 | 0.4 | 30 | 70 |
| 7 | 0.4 | 30 | 70 |
| 15 | 0.4 | 70 | 30 |
| 16 | 0.4 | 70 | 30 |
| 30 | 0.4 | 10 | 90 |
| 33 | 0.4 | 10 | 90 |

## Results and discussion

### Antimicrobial compounds and detection frequencies

The results of the screened samples for the presence of 48 antimicrobial compounds were summarised in Figure 2, where the number of contaminated samples at a higher and lower MRLs value was represented in each matrix.

Among 99 milk samples analysed, 34 were contaminated by antimicrobials. The detected compounds were presented in the Figure 2(a). Lincomycin, tylosine, and sulphamethazine were detected below their MRLs levels (150, 50 and 100 µg/kg) respectively. While oxytetracycline, sulfaquinoxaline and ciprofloxacin were found at a concentration higher than 100 µg/kg and cloxacillin at 30 µg/kg. Sulphonamide was the most detected antimicrobial compound in milk samples showing six active compounds (sulfamonomethoxine, sulfachinoxaline, sulfamethizole, sulphamethazine, sulfadoxine, and sulphadiazine).

From 155 antimicrobial compounds 83 chicken samples were contaminated (Figure 2(b)). Sulfaquinoxaline and tilmicosin were mostly reported in 40 and 28 chicken samples, respectively. Tylosin was detected in 2 samples at concentrations higher than the MRL (50 µg/kg). The level of contamination in the analysed chicken samples by oxytetracycline, tetracycline, chlortetracycline and doxycycline, which belong to the tetracyclines, shows their large use in the medications, which is also corroborated by Bion et al. (2016). Ciprofloxacin and enrofloxacin were found in chicken

**Table 2.** MRM transitions, dwell time, fragmentation and collision energy for quinolones, sulphonamide, macrolides, β-lactames, aminoglycosides, lincosamides, and tetracyclines.

| Compound | Precursor ion | Product ions | Dwell (s) | Fragm. (V) | Coll. En. (V) |
|---|---|---|---|---|---|
| Amoxycillin | 366.1 | 349.3 | 10 | 70 | 5 |
| | | 210.9 | | | 10 |
| Ampicillin | 350.1 | 191.8 | 10 | 110 | 10 |
| | | 106 | | | 20 |
| Apramycin | 540.3 | 378 | 5 | 90 | 15 |
| | | 217 | | | 25 |
| Cefquinone | 529.1 | 324 | 10 | 40 | 15 |
| | | 134.2 | | | 5 |
| Cephalaxine | 348.1 | 158 | 40 | 80 | 5 |
| | | 106 | | | 30 |
| Chlortetracyclines | 479.1 | 462.1 | 5 | 60 | 15 |
| | | 444.1 | | | 20 |
| Ciprofloxacine | 332.1 | 314 | 5 | 100 | 20 |
| | | 288.1 | | | 15 |
| Clavulanic acid | 197.2 | 178.9 | 5 | 80 | 15 |
| | | 148.9 | | | 20 |
| Cloxacillin | 436.1 | 276.9 | 10 | 60 | 5 |
| | | 159.8 | | | 5 |
| Danofloxacine | 358.1 | 340.1 | 5 | 100 | 20 |
| | | 314.1 | | | 15 |
| Demeclocycline (IS) | 464.9 | 44 | 15 | 140 | 20 |
| Dicloxacillin | 470 | 310.9 | 10 | 80 | 15 |
| | | 160.1 | | | 5 |
| Difloxacine | 400.1 | 356.1 | 5 | 70 | 20 |
| | | 299.1 | | | 30 |
| Dihydrostreptomycin | 584.3 | 262.9 | 5 | 120 | 30 |
| | | 245.9 | | | 35 |
| Doxycycline | 445.1 | 428.2 | 5 | 60 | 15 |
| | | 154 | | | 15 |
| Enrofloxacine | 360.2 | 316.2 | 5 | 70 | 20 |
| | | 245.2 | | | 30 |
| Flumequin | 262 | 242 | 5 | 70 | 15 |
| | | 202 | | | 15 |
| Erythromycin | 734.4 | 576.3 | 5 | 60 | 15 |
| | | 157.9 | | | 30 |
| Gentamycin | 478.3 | 322.2 | 5 | 120 | 10 |
| Gentamycin | 464.3 | 322 | 5 | 120 | 10 |
| Gentamycin | 450.1 | 322.1 | 5 | 120 | 10 |
| Josamycin | 828.4 | 173.9 | 5 | 70 | 30 |
| | | 109 | | | 30 |
| Kanamycin | 485 | 324.2 | 5 | 60 | 15 |
| | | 163.2 | | | 5 |
| Lincomycin | 407.2 | 359.3 | 5 | 70 | 15 |
| | | 126 | | | 30 |
| Nafcilin | 415.1 | 198.8 | 10 | 110 | 5 |
| | | 170.8 | | | 40 |
| Nalidixic acide | 232.9 | 214.8 | 5 | 70 | 5 |
| | | 186.8 | | | 25 |
| Neomycine | 615.3 | 292.9 | 5 | 120 | 25 |
| | | 160.9 | | | 30 |
| Norfloxacillin | 320.2 | 302.1 | 5 | 110 | 20 |
| | | 275.9 | | | 15 |
| Oxolinic acid | 262.1 | 243.9 | 5 | 40 | 15 |
| | | 215.9 | | | 30 |
| Oxytetracycline | 461.1 | 443.1 | 5 | 70 | 5 |
| | | 425.9 | | | 20 |
| Paromomycine | 616.3 | 324.1 | 5 | 100 | 20 |
| | | 162.9 | | | 40 |
| Penicillin G | 335 | 289.1 | 10 | 100 | 25 |
| | | 128 | | | 25 |
| Penicillin V | 351 | 160 | 10 | 40 | 5 |
| | | 114 | | | 40 |
| Sarafloxacine | 386 | 342.1 | 5 | 60 | 20 |
| | | 299.1 | | | 30 |
| Spectinomycine | 351.1 | 333.1 | 5 | 120 | 15 |
| | | 207.1 | | | 20 |
| Spiramycin | 422.4 | 173.9 | 5 | 80 | 20 |
| | | 100.9 | | | 15 |
| Streptomycine | 582.2 | 263.1 | 5 | 120 | 35 |
| | | 245.9 | | | 35 |
| Sulphadiazine | 251.1 | 155.9 | 5 | 60 | 10 |
| | | 108 | | | 25 |
| Sulfadimethoxime | 311.1 | 156 | 5 | 60 | 20 |
| | | 108 | | | 30 |
| Sulfadoxine | 311.1 | 156 | 5 | 90 | 15 |
| | | 108 | | | 25 |
| Sulfadoxin-d3 (IS) | 314.1 | 156 | 15 | 60 | 30 |
| Sulfaphenazole (IS) | 315.1 | 157.9 | 5 | 60 | 30 |
| Sulfaguanidine | 215.1 | 156 | 5 | 80 | 10 |
| | | 108 | | | 20 |
| Sulfamethoxypyridazine | 281.1 | 156 | 5 | 60 | 20 |
| | | 108 | | | 30 |
| Sulfamonomethoxine | 281.1 | 215 | 5 | 110 | 15 |
| | | 156 | | | 15 |
| Sulfaquinoxalin | 301.1 | 156 | 5 | 80 | 15 |
| | | 108 | | | 25 |
| Sulphathiazole | 256 | 156 | 5 | 40 | 10 |
| | | 108 | | | 20 |
| Sulphamethazine | 279 | 186 | 5 | 60 | 15 |
| | | 156 | | | 15 |
| Sulphamethizole | 271 | 155.9 | 5 | 60 | 10 |
| | | 107.9 | | | 25 |
| Tetracycline | 445.1 | 427.2 | 5 | 100 | 5 |
| | | 410.1 | | | 20 |
| Tilmicosin | 869.5 | 173.9 | 5 | 70 | 40 |
| | | 131.9 | | | 40 |
| Tylosin | 916.5 | 772.5 | 5 | 110 | 30 |
| | | 173.8 | | | 40 |

IS: internal standards.

samples at concentrations higher than the MRL of 100 µg/kg, as shown by Rasheed et al. (2017). These compounds showed damage to the juvenile joints, the kidneys, the eyes, and the central nervous system, which has been previously reported by animal experiments (Patterson 1991). Some antibiotic-induced allergic reactions have also been reported, in relation to quinolones (Blanca-Lopez et al. 2011).

Figure 2(c) shows that in beef less samples were contaminated by antimicrobials when compared to milk and chicken samples. Only 9 of 56 analysed samples contained authorised residues at levels lower than MRLs. Oxytetracycline, chlortetracycline, paromomycin, and sulfaquinoxaline were found at lower levels than the MRLs. In addition, oxytetracycline and sulfaquinoxaline were frequently detected in all studied matrices (chicken, beef, and milk). These results are in accordance with the most common drugs used in livestock (Cháfer-Pericás et al. 2010).

Table 3 shows that sulphonamides and tetracyclines represent the highest percentage of detected compounds s with 44% and 26%, respectively, above the MRLs in the analysed samples. Furthermore, β-Lactams and macrolides were also observed above the MRLs, at the percentage of 6% to 10% in the samples. Moreover, aminoglycosides were detected below the MRLs in 3%

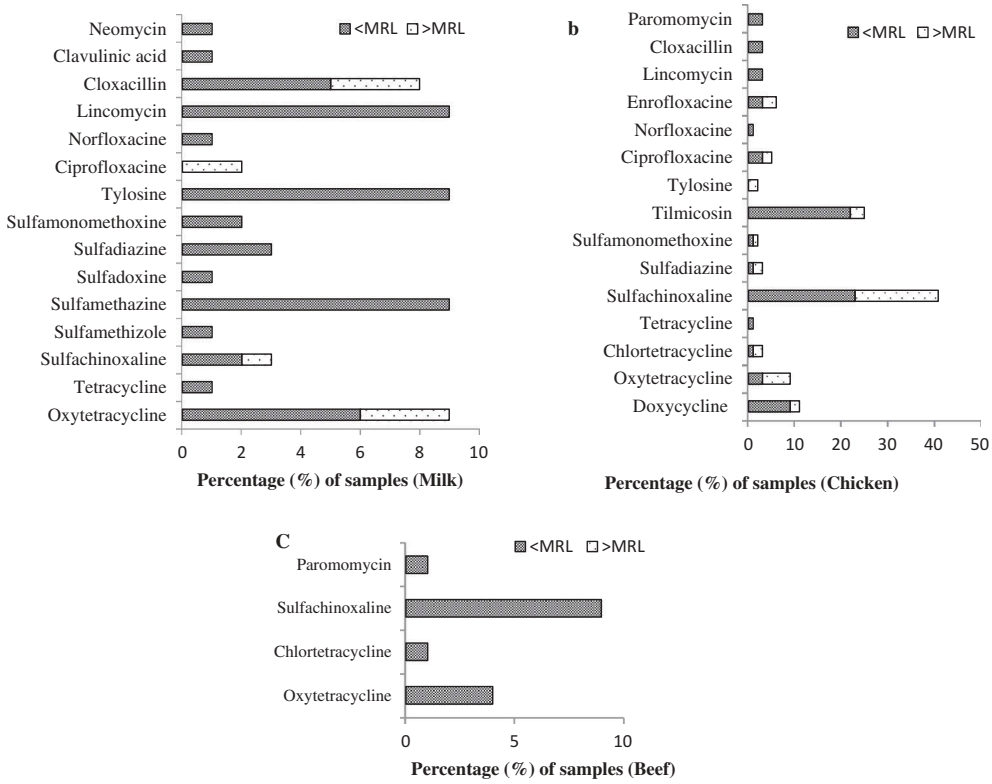


Figure 2. Distribution of antibiotics in milk, chicken and beef according to the MRLs.

Table 3. Percentage of positive samples, with concentrations below or above the MRLs.

| | Samples | |
|---|---|---|
| Category | <MRL | >MRL |
| Tetracyclines | 17% | 26% |
| Sulphonamides | 35% | 44% |
| Macrolides | 23% | 10% |
| Quinolones | 6% | 14% |
| Lincosamides | 9% | 0 |
| B-Lactams | 7% | 6% |
| Aminoglycosides | 3% | 0 |

of the samples. Comparing our results with corresponding bibliographic data of other countries such as Germany, Kenya and the Netherlands, sulphonamides, and tetracyclines were the most common families detected above the standard residual limits in food-producing animals (Orwa et al. 2017). Moreover, these consider the attention, due to their stability for a long time when compared to other compounds of investigation (Kim et al. 2013).

## *Multiple residues in analysed samples*

In 69% of the milk samples no antimicrobial residues were found above the limit of detection related to each compounds (5–10 µg/kg). Therefore, 4% of the milk samples were contaminated with four residues in total. Moreover, 1 or 2 antimicrobial compounds were revealed in 17% and 13% of the milk samples, respectively. The presence of more than one compound s in milk samples can be explained by the use of antimicrobials to treat clinical diseases, to prevent and control common disease events, and to enhance animal growth. For example, 16% of all lactating dairy cows in the U.S. receive antimicrobials therapy for clinical mastitis each year, but nearly all dairy cows receive intermammary infusions of prophylactic doses of antimicrobials following each lactation to prevent and control future mastitis (Landers et al. 2012). In addition, 15% of beef calves in the US that enter feedlots receive antimicrobials for the treatment of clinical respiratory disease, but therapeutic antimicrobials are also administered to 10% of apparently healthy calves to mitigate anticipated outbreaks of respiratory disease (Landers et al. 2012).

In chicken, 47% of the analysed samples show no antimicrobials residues, but 1% only contain four residues in the same sample. More recent estimates by the Union of Concerned Scientists, an advocacy group that supports reduced agricultural antimicrobial use, suggest that 24.6 million pounds of antimicrobials are used for nontherapeutic purposes in chicken, cattle, and swine in US. In Lebanon, the estimation of the antimicrobials abuse in livestock was exempt. Twelve

classes of antimicrobials may be used at different times in the life cycle of poultry, cattle, and swine (Landers et al. 2012). This fact explains the high detection of antimicrobials contamination in the chicken samples and the detection for more than one active compound in the same sample.

Moreover, the percentage of beef samples that contain zero residues was 84%. These results confirm that chicken matrices are more contaminated with antimicrobial residues compared to beef samples. Our results emphasise the necessity to investigate the potential presence of antibiotic residues in food and the adverse health risks associated with the development of antimicrobial-resistant pathogens.

## Antimicrobials residues in Lebanese governorate

Six important Lebanese cities from different Lebanese governorates were chosen to be screened for the presence of antimicrobial residues in their food-producing animal. This is a representative area in the field of livestock production in Lebanon. Chtoura, Akkar, and Hermel were characterised by the presence of an important number of industries for dairy products. In recent years, livestock production (goats and sheep) has relied increasingly on feed blocks and feed supplements, thereby reducing dependence on wild grazing and ultimately leading to more sedentary animal production. Bovines and dairy production are becoming increasingly popular. In the past 5 years, several medium-to large-scale dairy farms have been established in the North and in Beqaa (Ministry of Environment 2007). Beqaa governorate is home to extensive dairy farming, covering 44% of the country's total farming land. Nearly 75–80% of Lebanon's cows, 45% of goats and 35% of sheep are raised in this region, which produces 188 tons of milk a day (Haddad and Chamoun 2014). The size of the dairy market in Lebanon is approximately 200 million dollars, with a total production estimated to be at 62,000 metric tons per year.

Chicken is present in almost every household's kitchen in Lebanon. Locally, Lebanon produces around 150 million kilos of broilers (chickens destined for meat consumption) and consumes 30 kg of chicken per capita per year. The country is home to more than 10 large poultry producers and some 2,000 poultry farms. The Lebanese poultry producers have the capacity to tend to the entirety of local demand.

Three hundred ten samples (milk, chicken, and beef) were collected from Mount of Lebanon, Hermel, Chtoura, Saida, Nabatieh, and Akkar in order to check their compliance with the European regulation (Commission decision 2002/657/EC). Table 4 shows the percentage of positive samples below and above the MRLs in each governorate for all matrices. In Mount of Lebanon, 56% of chicken samples were contaminated by residues of antimicrobial at below the MRLs, compared to 19% and 16% for milk and beef samples, respectively. In this area, 4% and 1% of the samples revealed a value above the MRLs for milk and chicken, respectively. Therefore, the other sites showed a lower percentage (1–15%) of contaminated samples for the three matrices below the MRLs (Table 4). Nabatieh may not report a higher percentage samples above the MRLs for chicken beef and milk and it fulfils the requirements of the European legislation. For milk, Akkar revealed a less percentage of samples (1%) contaminated by antimicrobials below the MRLs when compared to Hermel and Chtoura. Regarding the percentage of contamination, the Mount of Lebanon will be considered the most contaminated for all matrices in comparison with the other sites. The difference of contamination between the governorates can be related to the level of expertise in the setting of the withdrawal times by the farmers.

Table 4. Distribution of positive samples of milk, chicken and meat, according to their concentrations compared to the MRLs and according to their origin in Lebanon.

| | Milk | | Chicken | | Meat | |
|---|---|---|---|---|---|---|
| Region | <MRL | >MRL | <MRL | >MRL | <MRL | >MRL |
| Hermel | 15 | – | 11 | 4 | 9 | 0 |
| Chtoura | 8 | – | – | 2 | – | – |
| Saida | 3 | – | 10 | 1 | – | – |
| Nabatieh | – | – | 6 | - | 9 | – |
| Akkar | – | – | 10 | 5 | – | – |
| Jabal lebnen | 19 | 4 | 56 | 1 | 16 | – |

Additionally, beef samples can be considered as less contaminated, because no samples were detected above the MRLs in all sampling areas. In contrast, the non-conformity in the chicken samples was detected in most sampling areas, which shows a widespread misuse of antimicrobials by poultry farmers and reflects a lack of implementation of withdrawal times. It is stressed that stricter regulation for inspection of chicken for residues prior to marketing and to evaluate the risk to human health according to difficulty in treating harmful resistant diseases.

## Discussion of the state of Lebanon

Considering the importance of consumer health towards antimicrobial residues in foodstuff, periodic sampling is carried out in many countries (Weiss et al. 2007; Zhao et al. 2009; Pena et al. 2010). Abdallah et al. (2014) reported 300 Lebanese meat samples (chicken and beef) and found

sulphonamides like sulphamethazine, sulfadimethoxine, sulfaquinoxaline, sulphadiazine, sulfamonomethoxie and sulfamethoxypyridazine residues in a rate of 16%. Despite this latter, no further studies of Lebanon regarding the contamination by seven antimicrobial families and the three different matrix beef, chicken, and milk were reported.

Regarding our results, 79% of the contaminated samples revealed the presence of antimicrobial residues below the MRLs, but also 21% above the MRLs. Most of these were contaminated by sulphonamide and tetracycline, that are also found in many other antimicrobial studies (Hiba et al. 2016; Hu et al. 2017). Nine per cent of the positive milk samples presented values above the MRLs and 52% below the MRLs for antimicrobial residues. Oxytetracycline, sulfaquinoxaline, ciprofloxacine, and cloxacillin were the most compounds found. Rama et al. (2017) showed that 6.1% of 1734 raw milk samples collected from individual farms and milk in six different major regions of Kosovo, contained positive drug residues with frequently detected residues of amoxycillin, penicillin G, and cloxacillin. Furthermore, tetracyclines (48.9%), sulphonamides (18.4%), and quinolones (6.8%) were found in milk samples from Macedonia below the MRLs. The presence of antimicrobial residues in milk can engender drug hypersensitivity reactions in milk consumers, manifested as dermal reactions, asthma, or anaphylactic shock (Rama et al. 2017). Competent authorities should establish and maintain continuous dairy monitoring programs to ensure risk-free milk products for Lebanese consumers. In addition, there is a need for additional research to accurately assess other aspects of this problem and designed to reduce milk contaminants.

Approximately 80% of the antimicrobial compounds sold in the world applied in meat and poultry production (Cháfer-Pericás et al. 2010). Most of the antimicrobial agents are used on healthy animals to promote growth, or to prevent diseases in crowded or unsanitary conditions. Nevertheless, their residues in food-producing animals are responsible for the modification of the intestinal flora (Marshall and Levy 2011).

On the other side, chicken samples presented a higher percentage of contamination (55%) compared to milk (37%) and beef (18%). This finding shows the critical situation in the Lebanese poultry sector. More than 27% of the chicken samples presented values above the MRLs, especially for sulfaquinoxaline (43% in positive samples). Studies on the occurrence of antimicrobials in poultry samples indicate higher rates of their residues in many other countries. Shareef et al. (2009) reported an antimicrobial residue rate of 52% in poultry products from 75 samples in Iraq. Amjad et al. (2005) analysed poultry products in Pakistan and reported 58% to 85% of the samples to contain ciprofloxacin residues and 55% to 92% of the samples contained enrofloxacin residues in violation of the regulations. Salehzadeh et al. (2007) and Farahmand et al. (2007) analysed 270 chicken samples from 90 broiler farms in Tehran, Iran, and found enrofloxacin at a value lower than 100 µg/kg. Enrofloxacin and ciprofloxacin were also detected in our study at 5% and 6% respectively, from the positive chicken samples. For beef samples, only 12% revealed antimicrobial residues below the MRLs, which was lower compared to values reported in other countries like Burkina Faso and Vietnam (Samandoulougou et al. 2015; Hoang Ngoc Do et al. 2016). The lower percentage of antimicrobial residues reflects that the withdrawal, the time period between the last dose of the drug given to animals and the consumption of animal food, has been respected before the abatement of the animals. The present result must be conserved and this adds pressure not only on livestock producers but also on management authorities in Lebanon to implement strict legislation with respect to the use of veterinary medicines.

## Conclusion

This study revealed the presence and the levels of contamination by 48 compounds from six families of antimicrobial compounds widely used in animal production. The study was based on 310 samples, including milk, chicken, and beef collected from different Lebanese regions. The results show that 40% of the analysed samples contained antimicrobial residues. The quantification results confirm that 21% of the samples are non-compliant and exceed the maximum residue limit set by the European Union. Sulphonamide and tetracycline showed the highest residue levels since they are the most applied in treating veterinary diseases. In addition, 27% of the chicken samples contained antimicrobial residues above the MRLs. The residue rates in chicken were significantly higher than the ones reported in milk (9% > MRLs) and beef (0% > MRLs). Oxytetracycline, doxycycline, enrofloxacin, ciprofloxacin, tilmicosin, sulfaquinoxaline, sulphamethazine, and sulfadimethoxine were the most detected residues in all matrices. These results showed indiscriminate and irrational use of antimicrobial agents, especially in the poultry sector, that may result in unwanted residues in animal food and could cause serious health hazards to consumers. Continued antimicrobial surveillance in the poultry sector may be essential. Hence, there is a need to respect the withdrawal periods of antimicrobials in order to reduce the level of antimicrobial residues in food samples to a minimum and to reinforce controls

through regular sampling and analysis with a larger number of samples and more varieties of matrices. The outcome of this study provides valuable basic information for local governmental authorities for effective monitoring of the use and misuse of agricultural antimicrobial agents. The obtained data will also be helpful for other Gulf Cooperation Council countries, because these countries share similar farming practices and import comparable foodstuffs.

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## Disclosure statement

All authors have approved the submission and none declare any conflict of interest in the work performed or in the submission of the manuscript.

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