Screening and quantification of antibiotic residues in broiler chicken meat and milk in the central region of Algeria

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Summary

Antibiotics used in animals may be found in food of animal origin and pose a risk to human health. The aim of this study was to screen for antibiotic residues in broiler chickens and milk. Two hundred and twenty-one samples were collected (71 samples of chicken breast meat, 117 samples of raw cow’s milk and 33 samples of raw goat’s milk). The chicken meat samples underwent a microbiological analysis, followed by a physical/chemical analysis using high-performance liquid chromatography (HPLC). The milk samples were screened using the Delvotest® SP microbiological test, followed by the BetaStar® Combo test for residues of beta-lactams and tetracyclines.
For chicken meat, 32.39% of the samples were positive, with 56.52% of these samples containing aminoglycosides, 52.17% containing sulphonamides, 30.43% containing beta-lactams and/or tetracyclines and 21.73% containing macrolides. The concentrations of amoxicillin, penicillin G, erythromycin and sulfisoxazole exceeded the maximum residue limits (MRL) laid down in European regulations in 28.57%, 85.71%, 80% and 91.66% of samples, respectively. The results of the Delvotest® SP test on milk showed that 12.6% of samples were contaminated by inhibiting substances. Beta-lactams and tetracyclines were present in 26.32% and 15.79% of the samples analysed, respectively. These results indicate that the contamination of chicken meat and milk is due to non-compliance with administration procedures and poor use of antibiotics.

**Keywords**


**Introduction**

Medicines and antibiotics form part of the therapeutic arsenal required for farming today and are used to prevent or treat a wide range of infectious diseases. Compliance with the procedures for their use ensures a very high likelihood of residues remaining below the maximum residue limit (MRL) (1). Conversely, failure to respect such procedures and the improper use of antibiotics can result in residue levels above the authorised thresholds for foodstuffs from treated animals. This can lead to microbiological, toxicological and allergenic hazards for consumers, as well as technological hazards for the agrifood industry (2).

Consumers throughout the world today are concerned about the safety and quality of the food they eat. However, control measures differ from one country to another. The prevalence of veterinary medicinal residues in foods of animal origin is below 1% in Europe, but as much as 94% in some African countries (3). Unlike Europe, in Algeria the presence of antibiotic residues in foods of animal origin has rarely attracted serious attention and few controls are conducted on distribution
channels. Screening for residues had never been part of a proper surveillance programme until 2013, with the advent of a new programme called the Algerian surveillance programme for contaminants and residues in food (PASCRA) launched by the Ministry of Agriculture, Rural Development and Fisheries and financed by the European Union (4).

Currently, no official national statistics are available on the prevalence of antibiotic residues, antibiotic consumption and use, or risk factors on the farm. Nevertheless, many recent studies have revealed multiple resistance to antibiotics in bacterial strains isolated in different species of livestock (5, 6, 7, 8).

The aim of this study was to screen for and quantify antibiotic residues in broiler chicken meat, raw cow’s milk and raw goat’s milk collected at points of sale and collection centres.

**Materials and methods**

**Sampling**

The study was conducted on 221 samples taken in wilayas (provinces) in central Algeria (Alger, Blida, Tipaza, Médéa, Ain Defla and Tizi Ouzou), which included:

- 71 chicken breasts bought at various points of sale (specialised poultry shops and supermarkets), either as whole chickens or cuts of chicken; the samples were selected randomly, at a rate of six samples a week;

- 117 samples of raw cow’s milk were taken aseptically from the taps of milk tanker trucks supplying a dairy factory in the wilaya of Blida as part of the company’s in-house quality control programme, at a rate of six samples a week;

- 33 samples of raw goat’s milk collected from refrigerated tanks on goat farms.
All samples of raw cow’s milk were transported to the laboratory under refrigeration (+4°C), while chicken meat and goat’s milk samples were frozen at –20°C prior to analysis.

**Materials**

Residues in chicken meat were screened for using the microbiological four-plate method. This test was used on a strain of *Bacillus subtilis* (ATCC 6633) (growth mediums with pH = 6, pH = 7.2 and pH = 8) and a strain of *Micrococcus luteus* (ATCC 9341) (growth medium with pH = 8). Control solutions were prepared containing a reference antibiotic or sulphonamide (penicillin G sodium, sulfathiazole, dihydrostreptomycin or erythromycin).

This was confirmed using a Shimadzu high-performance liquid chromatography machine with a column (LiCrospher® 100 RP-18 [5 μm] in reversed phase: 46 mm × 250 mm) and a visible ultraviolet (UV) detector incorporated in the chromatography system (of the same type) to quantify the concentration of antibiotic residues.

To screen for residues in milk, the authors used well-known and proven commercial tests: the Delvotest® SP kit (broad-spectrum microbiological qualitative test) and the BetaStar® Combo test (qualitative and semi-quantitative test, beta-lactam- and tetracycline-specific).

**Methods**

**Screening for antibiotic residues in chicken breast meat**

*Microbiological method (four-plate test)*

This is an official method for checking meat from livestock and poultry. It uses agar gel diffusion to reveal residues of antibiotic substances belonging to the beta-lactam, tetracycline, sulphonamide, aminoglycoside and macrolide families (9).
Confirmation by a quantitative method (high-performance liquid chromatography)

Positive samples of certain residues were identified and quantified using a physical/chemical method: high-performance liquid chromatography (HPLC). Before being identified and quantified, the antibiotic residues were extracted from their matrices according to protocols developed by Lee et al. (10). The experimental conditions for separation (mobile phase, $\lambda_{\text{max}}$ detection, throughput, volume injected) are described by Lee et al., as well as in an official French food safety agency (AFSSA) document (11).

Screening was carried out for the antibiotics most frequently used in Algeria, namely beta-lactams (amoxicillin, ampicillin, penicillin G and oxacillin), a sulphonamide (sulfisoxazole) and a macrolide (erythromycin).

In the absence of national regulations, the samples analysed were classified according to the MRLs defined in European regulations (12).

Screening for antibiotic residues in milk

The authors used the Delvotest® SP test to screen all samples, followed by BetaStar® Combo to search for residues of beta-lactams and tetracyclines in positive milk samples. The manufacturer’s instructions concerning the protocol and final interpretation were followed scrupulously.

Statistical analysis

The results are presented as percentages (number of positive samples/samples analysed) with confidence intervals of 95% (CI = 95%), calculated using SYSTAT software, version 10.
Results

Screening for residues in chicken breast meat

Microbiological method

Out of 71 samples of chicken meat, 23 tested positive, equal to 32.39% [CI = 95%: 22.66–43.94]. The results of screening for different groups of antibiotics are given in Table I. The majority of positive samples were from sulphonamides and aminoglycosides.

Table I
Number and rate of positive chicken meat samples detected by microbiological analysis

<table>
<thead>
<tr>
<th>Families of antibiotics</th>
<th>Positive samples (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (a)</td>
</tr>
<tr>
<td>Beta-lactams and/or tetracyclines</td>
<td>7</td>
</tr>
<tr>
<td>Beta-lactams and/or macrolides</td>
<td>5</td>
</tr>
<tr>
<td>Sulphonamides</td>
<td>12</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>13</td>
</tr>
</tbody>
</table>

CI: confidence interval
a) a sample may be positive for several families of antibiotics simultaneously

Table II shows the presence of a single family of antibiotics (mono-contaminated samples), or of several families in the same sample (poly-contaminated samples).

Table II
Number of families of antibiotics contaminating chicken meat samples

<table>
<thead>
<tr>
<th>Positive samples (n = 23)</th>
<th>No. of families of antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One</td>
</tr>
<tr>
<td>No. of samples</td>
<td>15</td>
</tr>
<tr>
<td>Percentage</td>
<td>65.2</td>
</tr>
</tbody>
</table>

CI: confidence interval
The majority of samples, representing 65.2% [CI = 95%: 44.89–81.19], were mono-contaminated. In the remaining 34.8% [CI = 95%: 18.81–55.11], the following combinations were found: aminoglycosides/sulphonamides, aminoglycosides/beta-lactams, aminoglycosides/macrolides and beta-lactams/macrolides.

**Confirmation by the quantitative method**

Table III shows screened residues confirmed by HPLC, from samples identified as positive using the microbiological method, and compared with their MRL.

**Table III**

Concentration of antibiotic residues detected in chicken meat using high-performance liquid chromatography

<table>
<thead>
<tr>
<th>Families of antibiotics</th>
<th>Residues screened</th>
<th>Concentrations (µg/kg)</th>
<th>MRL(a) (µg/kg)</th>
<th>Samples in excess of MRL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-lactams and/or tetracyclines (n = 7 samples)</td>
<td>Amoxicillin</td>
<td>61.79</td>
<td>381.95</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Ampicillin</td>
<td>12.16</td>
<td>19.13</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Penicillin G</td>
<td>97.64</td>
<td>368.85</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Oxacillin</td>
<td>–</td>
<td>–</td>
<td>300</td>
</tr>
<tr>
<td>Beta-lactams and/or macrolides (n = 5 samples)</td>
<td>Erythromycin</td>
<td>127.26</td>
<td>334.53</td>
<td>200</td>
</tr>
<tr>
<td>Sulphonamides (n = 12 samples)</td>
<td>Sulfisoxazole</td>
<td>98.01</td>
<td>912.14</td>
<td>100</td>
</tr>
</tbody>
</table>

MRL: maximum residue limit

a) European Commission Regulation (EU) no. 37/2010 (12)

The predominant beta-lactam family residue was penicillin G (85.71%). A point of note is that concentrations of all the molecules tested, with the exception of ampicillin and oxacillin, exceeded their MRL.
The concentrations of amoxicillin, penicillin G, erythromycin and sulfisoxazole exceeded their MRL in 28.57%, 85.71%, 80% and 91.66% of quantified samples, respectively.

**Screening for antibiotic residues in milk**

**Screening by Delvotest® SP**

The results of screening for inhibitor residues using the Delvotest® SP test are given in Table IV. Of the 150 milk samples analysed, 19 tested positive, equal to 12.67% [CI = 95%: 8.26–18.94].

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of samples</th>
<th>Positive samples</th>
<th>Negative samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>CI = 95%</td>
<td>No. (%)</td>
</tr>
<tr>
<td>Cow’s milk</td>
<td>117</td>
<td>14 (11.97)</td>
<td>103 (88.03)</td>
</tr>
<tr>
<td>Goat’s milk</td>
<td>33</td>
<td>5 (15.15)</td>
<td>28 (84.85)</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>19 (12.67)</td>
<td>131 (87.33)</td>
</tr>
</tbody>
</table>

CI: confidence interval

**Screening using BetaStar® Combo**

The results of screening for residues of beta-lactams and tetracyclines using BetaStar® Combo, on positive samples screened using Delvotest® SP, gave the following respective rates: 21.43% [CI = 95%: 7.57–47.59] and 14.29% [CI = 95%: 4.01–39.94] for cow’s milk and 40% [CI = 95%: 11.76–76.93] and 20% [CI = 95%: 3.62–62.45] for goat’s milk (Table V).
Table V
Screening for residues of beta-lactams and tetracyclines in milk using BetaStar® Combo

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of samples</th>
<th>Beta-lactams</th>
<th>Tetracyclines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive no. (%)</td>
<td>Negative no. (%)</td>
</tr>
<tr>
<td>Cow's milk</td>
<td>14</td>
<td>3 (21.43)</td>
<td>11 (78.57)</td>
</tr>
<tr>
<td>Goat's milk</td>
<td>5</td>
<td>2 (40.00)</td>
<td>3 (60.00)</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>5 (26.32)</td>
<td>14 (73.68)</td>
</tr>
</tbody>
</table>

Discussion

Screening for antibiotic residues in chicken meat found a contamination rate of 32.39%. The targeted microbiological method revealed that 56.52% of samples contained aminoglycosides, 52.17% contained sulphonamides, 30.43% contained beta-lactams and/or tetracyclines and 21.73% contained macrolides.

The presence of antibiotic residues in these samples could be the result of a number of different factors, including the abusive use of antibiotics by professionals for curative, prophylactic or metaphylactic purposes. The presence of sulphonamides probably results from their frequent use as an additive in poultry feed to prevent coccidiosis (13, 14), which is common on Algerian farms (15).

According to a French study in 2015 (16), the results of a survey to monitor residues in poultry and eggs revealed non-conformities for the antibiotics oxytetracycline and sulfadimethoxine in meat from poultry other than broiler chickens (ducks, spent laying hens). Farm inspections showed that these non-conformities resulted from the use of medicated feed (16). It is worth noting that the species most exposed to antibiotics are poultry, rabbits, pigs and veal calves, because they are raised in groups indoors and are, therefore, subject to considerable infection pressure (17). Some authors believe that the presence of residues in feed...
is linked to poor farming practices and anarchic distribution of medicinal products by farmers who also totally ignore the rules for administering these products (18). A survey among veterinarians working on farms in eastern Algeria found that only 15% of practitioners believed that farmers respected the correct waiting period (19).

The results of the present study also showed that 34.8% of positive samples were poly-contaminated, with the most notable combinations being aminoglycosides/sulphonamides, aminoglycosides/beta-lactams, aminoglycosides/macrolides and beta-lactams/macrolides. This could be the result of repeated therapeutic interventions using different molecules or by the farmer administering medication unadvisedly.

Quantification of these residues revealed rates well in excess of their MRLs, with values more than seven times the limit for amoxicillin and penicillin G, more than one and a half times for erythromycin, and more than nine times for sulfisoxazole. These results probably indicate failure to comply with procedures for the use of antibiotics (dosage, route of administration and, above all, waiting period), all procedures aimed precisely at ensuring a high probability of residue levels below their defined MRL.

It is important to note that a lack of monitoring programmes in developing countries leads to non-compliance with animal health measures and the irrational use of veterinary medicinal products (including prohibited products), resulting in very high levels of contamination. A study by Karmi in Egypt (20) reported that 90%, 88% and 72% of fresh products, local frozen products and imported frozen products, respectively, were contaminated by antibiotic residues. Tavakoli et al. in Iran (21) reported contamination levels of 88% in poultry samples tested for chloramphenicol, a drug that is prohibited in veterinary medicine.

Screening for antibiotic residues in milk using Delvotest® SP found that 12.67% of milk samples were contaminated by inhibiting substances. These substances can be endogenous in origin, such as lysozyme, lactoferrin and lactoperoxidase, or exogenous, mainly from antibiotics
Antibiotic therapies, which play a major role in the treatment and prevention of mammary infections, are the main source of antibiotics in milk. It has been estimated that the intra-mammary route is responsible for 72% of the antibiotics found in milk (22).

BetaStar® Combo screening for beta-lactams and tetracyclines revealed positive results of 26.32% and 15.79% respectively for the two families.

Beta-lactams and tetracyclines are certainly the most active and most frequently used in clinical treatment, which explains their inevitable presence in milk compared with other molecules. The results of a survey by Ameur et al. on the use of intra-mammary antibiotics by veterinarians practising in the Kabylie region found that the most frequently used products were tetracyclines, penicillins and, more rarely, macrolides (23). Other surveys carried out by Tarzaali et al. on screening for residues in cow’s milk in Algeria reported positive results of 65.46% and 89.09% for beta-lactams and tetracyclines, respectively (24).

The presence of antibiotic residues in food is not without consequences; its effects can be seen in humans and the environment when antibiotics enter water and the ground via the excrement of treated animals, either in their original state or in the form of metabolites (25, 26). Apart from their repercussions on health, residues in food can affect international trade, under the terms of the World Trade Organization (WTO) agreement on sanitary and phytosanitary measures (SPS agreement) (3).

International monitoring of veterinary medicinal product residues in food of animal origin differs from one country to another in terms of the regulations in force.

In Algeria, there is a lack of official data from the veterinary authorities; data on antibiotic residues in food of animal origin are restricted to a few research efforts or students’ final dissertations. The results of research undertaken as part of the PASCRA programme on veterinary medicinal products, anabolic substances, heavy metals and microbiological contaminants, have yet to be published. The
implementation of such a programme should guarantee food safety for Algerian consumers equivalent to that in developed countries and allow products of animal origin to be exported to international markets.

**Conclusion**

This study reveals the presence of certain antibiotics in chicken meat and milk. Quantification of these products found concentrations that substantially exceeded the maximum limits laid down in European regulations, in the absence of Algerian standards. This highlights the potential danger to Algerian consumers. It is therefore imperative to implement systematic surveillance of antibiotic residues in food of animal origin, which is the only means of prevention that fully guarantees the safety of these food products. This requires reliable and specific detection and confirmation methods with appropriate detection and quantification thresholds, as well as an official definition of an acceptable residue level in food of animal origin. It is also very important to alert veterinarians and farmers to the dangers of the improper and uncontrolled use of such medicinal products.

**References**


