

## RESEARCH REGARDING THE QUALITATIVE ASSESSMENT OF THE CONTAMINATION WITH ANTIBIOTIC RESIDUES FROM THE MILK SOLD IN THE AGRO-FOOD MARKETS AND IN THE COMMERCIAL NETWORK IN IAȘI

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### Abstract

The marketing of antibiotics directly to animal owners and the intensification in recent years of the use of these drugs without the recommendation of a veterinarian has led to the increasingly frequent presence of antibiotic residues in milk that is intended either for human consumption or for obtaining dairy products. The use by processors of milk with antibiotic residues is difficult to achieve. According to European Union food safety legislation, milk harvested from cows treated with antibiotics must be collected separately, and isolated. It cannot be given for processing or human consumption. Applying thermal treatment, especially pasteurization, to milk with antibiotic residues above the maximum allowed limit has insignificant effects, processors have been convinced of this over the years. The interest of the study for the detection of antibiotic residues in milk is very current, even if the waiting period after treating the cows with antibiotics is recommended, often this period is not respected, the consideration being the significant economic losses. The rapid strip test method was done with strip tests AuroFlow, which are designed for the simultaneous detection of beta-lactams and tetracyclines. The working samples were represented by processed milk sold in supermarkets and raw milk sold in agro-food markets in Iasi. The distribution of processed milk samples was 1 positive sample each in 2 of the supermarkets where the samples were collected, these being in the percentage of 4%. UHT-treated milk in all 5 supermarkets did not show any positive tests. On the other hand, the sale of raw milk in the agro-food markets showed a percentage of 20% positive samples, out of several 25 samples examined, the rate is much higher compared to processed milk.

**Keywords:** raw milk, processed milk, antibiotic residues, qualitative assessment

A wide range of antibiotics is used in the treatment of infectious diseases in cows, including the treatment of mastitis. The marketing of antibiotics directly to animal owners and the intensification in recent years of the use of these drugs without the recommendation of a veterinarian has led to the increasingly frequent presence of antibiotic residues in milk that is intended either for human consumption or for obtaining dairy products. The use by processors of milk with antibiotic residues is challenging to achieve.

Milk with antibiotic residues, even if it is harvested from a small number of cows, can have significant implications in obtaining dairy products based on fermentation processes, but also in the occurrence of allergies and antibiotic resistance in consumers (Saraz I., *et al*, 2017, Schlemper V., Sachet A.P., 2017).

Abusively and excessively used antibiotics easily facilitate the contamination of animal

products, especially milk, a product that is harvested daily from cows. When the maximum residue limit (MRL) is exceeded, in the case of antibiotics, it becomes a public health problem because of the reduced effectiveness of antibiotics in consumers (Sachi S., *et al*, 2019).

According to European Union food safety legislation, milk collected from cows treated with antibiotics must be collected separately, isolated, and cannot be given for processing or human consumption (Reg. 853/2004/EC). The application of heat treatment, especially pasteurization, to milk with antibiotic residues above the maximum allowed limit has insignificant effects, processors have been convinced of this over the years (Schlemper V., Sachet A.P., 2017).

The National Veterinary Sanitary and Food Safety Authority (ANSVSA) - through specialized directions, participate in the elaboration of the Plan National for the Control of Residues that transposes for the group of substances B1-

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antibiotics, the substances, and matrices are selected so that the entire range of antibiotics is covered, as well as according to the best-selling veterinary medicinal products on the Romanian market, taking into account that the maximum limits of residues to comply with Regulation (EC) no. 37/2010 and EURL Guidance on minimum method performance requirements (MMPRS) or specific pharmacologically active substances in specific animal matrices - 2020, thus the control and monitoring of residues guarantees more safety for consumers.

## MATERIAL AND METHOD

Investigations regarding the presence of antibiotic residues in milk were carried out between May 2021 and September 2022. The milk samples were collected from the commercial network, respectively from 5 supermarkets of different brands, in Iasi County. 5 samples of pasteurized milk and 5 samples of UHT milk were collected from each supermarket. The milk samples from the commercial network were of different brands and the pasteurized ones were kept at refrigeration temperature, at 4°C.

Samples of pasteurized milk and UHT milk had different percentages of fat, ranging from 3.8 to 1.5. During the study, there were a total of 25 samples of pasteurized milk, and 25 samples of UHT milk.

The second category of samples was represented by raw milk sold in 3 agro-food markets in Iasi county and raw milk sold on the street. Raw milk samples were collected in a number of 25, for these milk samples we did not have information regarding the percentage of fat contained in each sample.

The processing of milk samples for the detection of antibiotic residues was carried out in the "Milk and derived products technology" laboratory within the Faculty of Agriculture, Technology of Processing Agricultural Products Specialization, of Iasi University of Life Sciences which is equipped with equipment corresponding to the determinations made on milk and derived dairy products.

The rapid strip test method was done with the AuroFlow BT Combo Strip Test strips, which are designed for the simultaneous detection of beta-lactams and tetracyclines. These rapid tests detect 15 beta-lactam antibiotics and 3 major tetracyclines in milk at or below EU maximum residue limits (MRLs). The test has increased sensitivity, and high reproducibility and the reading of the results is done in 7 minutes.

The working protocol consisted in adding 0.2 ml of milk, with the help of a single-dose pipette, into the reagent wells, homogenizing by repeated aspiration with the pipette 10 times so that the lyophilized reaction particles were

completely dissolved in the milk. After 3 minutes of incubation at room temperature, I inserted the test strip into the well containing the sample to be examined. The waiting time for the reaction on the strip was 4 minutes, and immediately after that, I removed the sample pad from the strip.

The interpretation of the results was done with the naked eye depending on the positive or negative control line: - if the test lines are more saturated (visible) than the control line, the sample is negative.

- if the test lines are less saturated or as saturated as the control, the sample is considered positive and has antibiotic concentrations equal to or higher than the maximum allowed.

- if the test lines are not visible, this indicates a much higher concentration of the indicated antibiotic groups in the sample.

## RESULTS AND DISCUSSIONS

Milk is a product of animal origin whose food safety starts from the farm, which is the responsibility of all those involved to avoid unwanted effects on consumers (Bishop J.R., With C.H., 1984). Along the food chain, the procedures and control mechanisms applied to food should present minimal risk to the consumer, but this risk cannot be brought to zero for any food, regardless of how well some legal regulations are applied or what effective control systems exist for that food.

As producers are not legally obliged to specify the antibiotic content of animal products on the label, they are difficult to avoid and detect by consumers. At the same time, widely used both in the treatment of some diseases and in the feeding of animals, antibiotics can lead to an increase in the number of bacteria resistant to treatment with these drugs in humans (Saraz I., *et al*, 2017).

Mastitis represent welfare, but also an economic problem in dairy farms, with implications of reducing the quantity but also the quality of milk. Farmers when faced with such situations frequently use antibiotics from the beta-lactam group (penicillin G, ampicillin, amoxicillin, oxacillin, various cephalosporins) and tetracyclines (oxytetracycline, doxycycline, and tetracycline) (Gajda A. *et al*, 2018).

The European Union (EU) defines residues as "pharmacologically active substances (whether they are active principles, receptors or degradation products) and their metabolites that remain in food obtained from animals that have been administered the veterinary medicinal products in question.

The interest in the screening study of antibiotic residues in milk is very current, even if a waiting period is recommended for dairy cows following treatment with these drugs, often this is

not respected due to the significant economic losses.

Mohamed A.M. *et al*, 2020 conducted a study in Benadir finding that depending on the source of milk from 50 milk samples collected from different farms 15 were positive samples for antibiotic residues, which indicated a percentage of 30%, and out of 50 samples collected from various markets, 9 were positive, representing a percentage of 18% of samples with antibiotic residues. This study showed that farmers do not take into account a strict record of the administration of antibiotics,

especially in cows from which collect the milk, have no knowledge of antibiotic residues, and do not respect the waiting period before the milk is sold or processed.

The study of the screening of antibiotic residues from the milk collected from some supermarkets located in Iasi county shows us a return of 1 positive sample in 2 of the 5 supermarkets from which the samples were collected. Positive samples were found in pasteurized milk (*table 1*).  
characteristic.

Table 1

**Distribution of positive samples from milk sold in supermarkets from Iași**

Milk samples	S1			S2			S3			S4			S5		
	Nr.s.	P	N	Nr.s.	P	N	Nr.s.	P	N	Nr.s.	P	N	Nr.s.	P	N
Pasteurized milk	5	1	4	5	0	5	5	0	5	5	1	4	5	0	5
UHT milk	5	0	5	5	0	5	5	0	5	5	0	5	5	0	5
Total	10	1	9	10	0	10	10	0	10	10	1	9	10	0	10

S- supermarket, Nr.s.- sampels number, P.-positive, N.- negative

UHT (ultra-high temperature) treated milk sold in all 5 supermarkets did not show any positive test, which could be an indication that these residues would be destroyed at sterilizing temperatures to a greater extent than pasteurization.

Bondoc I., 2007, shows that in relation to the thermal treatment of milk with antibiotic residues, does not cause the destruction of antibiotics or produces insignificant reductions,

instead, sterilization can destroy a proportion of 20-60% of some antibiotics such as penicillin, streptomycin or aureomycin.

The milk sold in the agri-food markets, but also the one exposed for street sale, does not benefit from rigorous and periodic control of these antibiotic residues, as shown by our study through which we identified a number of 5 positive samples out of a total of 25 collected samples (*table 2*).

Table 2

**The distribution of positive samples from the milk sold in the agro-food markets in Iasi**

Milk samples	M. agro-food 1			M. agro-food 2			M. agro-food 3			Sold on the street		
	Nr. sampels	P	N	Nr. sampels	P	N	Nr. sampels	P	N	Nr. sampels	P	N
Raw milk	8	2	6	5	1	4	5	0	5	7	2	5

M.agro-alim – the agri-food market, P.-positive, N.- negative

Reporting as a percentage in supermarkets, the percentage of positive samples was 4% of the total of 50 samples collected and in the agro-food markets the percentage was much higher, being 20% of positive samples, but this percentage was related to half of the samples compared to supermarkets, respectively 25 samples.

The difference in percentages between thermally processed milk and raw milk sold in the markets is explained by the easy access of animal

owners to veterinary drugs without medical prescription and the lack of adequate control in the markets of these residues.

A study conducted by Kaya S.E., *et al*, in 2010 on milk samples collected from different points of sale in Ankara, indicated a percentage of 1.25%, being exceeded the maximum residual limits (MRL) for penicillin G, oxytetracycline, gentamicin, neomycin.

Table 3

**Distribution of positive samples according to the type of residues in the milk examined**

The type of residue	Pasteurized milk–positive	UHT milk-positive	Raw milk-positive
Beta-lactams	1	0	3
Tetracyclines	1	0	2
Total	2	0	5

The presence of antibiotics in raw milk can also be caused by the intentional addition of antibiotics to the milk by the farmer or animal owners to fake a high total germ count (NTGMA) milk from unhygienic milking, which involves the use of milk that has poor hygienic quality (Saraz I. *et al*, 2017).

The beta-lactam residues found in the 4 milk samples indicate a general injection treatment, this group containing a greater variety of antibiotics, and the 3 positive samples for tetracycline residue indicate a local treatment at the level of the mammary gland with intramammary syringes, these frequently containing tetracycline (table 3).

Tian L. *et al*, in 2016 demonstrate the resistance of drug residues to heat treatments used on milk and show that pasteurization of milk at 72°C is ineffective in removing these residues.

### CONCLUSIONS

The findings of this study indicated that market and street vendors of raw milk do not have the necessary knowledge about the impact of antibiotic residues on human health. At the same time, we can raise an alarm that constant exposure for long periods can lead to the phenomenon of antibiotic resistance in consumers. Regional and regional farmers can be trained on how to use antibiotic-based medicines so that they can be aware that a single milk can with antibiotic residues can contaminate a tank. The reduction of antibiotic residues in milk could be achieved by effective monitoring of the presence of teats in cows, and by using biologically active natural products with antibacterial and anti-inflammatory properties, thus it is possible to intervene in the food safety chain and reduce the use of antibiotics.

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