

Determination of tetracyclin and streptomycin residues by means of ELISA in pasteurized and UHT-sterilized milk^{*})

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Summary

Antibiotics are compounds widely used for their preventive qualities which, in turn, have beneficial results in production increase. However, as a result of unintentional or intentional procedures, limited quantities of antibiotic residues may remain in organisms and can cause problem in terms of human health and production of fermented milk products.

Streptomycin and tetracycline are the most common compounds displaying antibiotic properties. This study analyzed 126 (52 pasteurized and 74 UHT-sterilized) packaged milk samples for streptomycin residues and 167 (72 pasteurized and 95 UHT-sterilized) packaged milk samples for tetracycline residue using the ELISA technique. The samples were collected from various sales outlets all over Istanbul, Turkey. Streptomycin was found in 98 (77.8 %) of 126 samples. Amongst these, only one UHT-sterilized milk sample was found to contain 263 ppb streptomycin exceeding the approved threshold level of 200 ppb introduced in the Turkish Food Codex. Tetracycline residues were found in 161 (96.4 %) of 167 milk samples at about 200 ppb, however, none of these samples contained residues exceeding the 100 ppb threshold level in the Turkish Food Codex.

The study demonstrated that all the milk samples (except for one) contained antibiotic residues below threshold level. For the sake of public health it is vital to take protective measures such as preventing the careless use of antibiotics and ensuring that regulations pertaining to the waiting period following antibiotic treatments on animals are adhered to, as well as regularly analyzing antibiotic residues in raw milk delivered to milk production plants.

Keywords: milk, antibiotic, streptomycin, tetracycline

Similar to other world countries it is illegal to add any additives to pasteurized and UHT-sterilized milk in our country (11, 13). Despite of this restriction, chemical residues originating in raw milk causes problems (15). Antibiotic residues left from the treatment of mastitis and other animal diseases are very common (11, 14).

Penicillins, penicillin-streptomycin combinations and tetracyclins are the most common compounds that are used in animal infectious disease treatments (17). But residues of these compounds have a negative effect on both consumer health and milk processing.

It's reported that milk containing antibiotic residues causes many health problems in sensitive people from allergic reactions to aplastic anemia and anafilactic shock (11, 24). Also these residues change intestinal

flora and cause resistant bacteria (7). It's also known that antibiotic residues in raw milk cause a significant economical loss during the milk processing (3, 16, 28).

In order to prevent the health risks; which occur from milk containing antibiotic residues, some legal precautionary measures have been implemented such as not selling the milk of animals; which have received treatment; for a determined period of time (24). Various methods have been devised to determine the presence of residues (4, 8, 10). The most sensitive of these measures are enzyme immunologic measures in which residues even at the ppt level can be determined (12, 20, 22, 25, 29).

In Turkey, mastitis is a prevalent disease and antibiotics are commonly used for its treatment. Because of this it may become an important problem in public health, since it can pass to raw milk and to pasteurized and UHT-sterilized drinking milk. This study aims to quantify residues of streptomycin and tetracyclin;

^{*}) This work was supported by the Research Fund of the University of Istanbul (Project number: 1351/280799).

Tab. 1. Findings of streptomycin residues analysis in pasteurized and UHT-sterilized milks

Milk kind	n	Streptomycin negative sample numbers and ratios	Streptomycin positive sample numbers and ratios	Distribution according to the concentration (in ppb) of streptomycin in positive samples					Number of samples have over limits* given in T.F.C.**
				< 50.0	50.1-100.0	100.1-150.0	150.1-200.1	> 200.0	
Pasteurized milk	52	12 (23.0%)	40 (77.0%)	30 (57.7%)	8 (15.9%)	1 (1.3%)	1 (1.3%)	0 (0.0%)	0 (0.0%)
UHT-Sterilized milk	74	16 (21.6%)	58 (78.4%)	26 (35.1%)	20 (27.0%)	10 (13.5%)	1 (1.3%)	1 (1.3%)	1 (1.3%)
TOTAL	126	28 (22.2%)	98 (78.4%)	56 (44.4%)	28 (22.2%)	11 (8.7%)	2 (1.6%)	1 (0.8%)	1 (0.8%)

Explanations: * limit value: 200 ppb; ** T.F.C.: Turkish Food Code

Tab. 2. Findings of tetracyclin residues analysis in pasteurized and UHT-sterilized milks

Milk kind	n	Tetracyclin negative sample numbers and ratios	Tetracyclin positive sample numbers and ratios	Distribution according to the concentration (in ppb) of tetracyclin in positive samples					Number of samples have over limits* given in T.F.C.**
				< 5.0	5.1- 10.0	10.1-15.0	15.1-20.0	> 20.0	
Pasteurized milk	72	0 (0.0%)	72 (100.0%)	28 (38.9%)	33 (45.8%)	5 (6.9%)	3 (4.2%)	3 (4.2%)	0 (0.0%)
UHT-Sterilized milk	95	6 (6.3%)	89 (93.7%)	61 (64.2%)	15 (15.8%)	9 (9.5%)	2 (2.1%)	2 (2.1%)	0 (0.0%)
TOTAL	167	6 (3.6%)	161 (96.4%)	89 (53.3%)	48 (28.7%)	14 (8.4%)	5 (3.0%)	5 (3.0%)	0 (0.0%)

Explanations: * limit value: 100 ppb; ** T.F.C.: Turkish Food Code

which are widely used in our country; in pasteurized and UHT-sterilized milk.

Material and method

A total 167 packaged milk samples (72 pasteurized, 95 UHT-sterilized); of which were produced by 14 different companies at different dates, were collected from various sale points in Istanbul, Turkey.

Method: Pasteurized milk samples were kept at -20°C and UHT-sterilized milk samples were kept at room temperature until they were analyzed. The samples were centrifuged for 15 minutes at 3500 g/minute then fat was removed from the milk by vacuum aspiration. These fat free milk samples were put in tubes and kept in deep freeze.

In order to quantify the tetracyclin in the samples, RIDASCREEN® Tetracyclin Enzyme Immune Assay Test Kit (R-Biopharm, R 3501) was used according to the manufacturers instructions. During the test, washing procedures were performed with Biotek ELX 50 apparatus and the quantification was performed by ELX 800 apparatus and RIDAWIN program.

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Results and discussion

Streptomycin residue was found in 98 (77.8%) of 126 milk samples (52 pasteurized, 74 UHT-sterilized) in a quantity reaching 263 ppb. Only one UHT-sterilized milk sample had residue at 263 ppb; which is over

the approved threshold of 200 ppb given in Turkish Food Code. Streptomycin quantities in UHT-sterilized and pasteurized milks are given in Table 1.

Tetracyclin was determined in 161 (96.4%) of 167 samples (72 pasteurized, 95 UHT-sterilized). The highest level of tetracyclin was 29.0 ppb. The findings about this analysis were given in Table 2.

Streptomycin and tetracyclin are two antibiotics widely used in animal treatments. Regarding the prevention of residues passing into milk, world countries have developed rules and regulations wherein waiting periods and maximum limit values have been determined (1, 18, 19, 26). Elimination duration of antibiotics for milk changes depending on application form, number of applications, dosage and type of antibiotic suspension (5). Waiting period is 6 days for streptomycin (23) and 5 days for tetracyclin (14). Maximum approved limit for residue quantity in milk are 200 ppb for streptomycin and 100 ppb for tetracyclin according to Turkish Food Code (1).

Although a variety of methods can be used regarding the determination of antibiotic residues in milk, ELISA was preferred in this study. Several studies have reported that ELISA is a quick, precise and practical method (21, 25).

According to the finding of this study, streptomycin residues were found in 77.8% and tetracyclin residues were found in 96.4% of the milk samples. But when an evaluation according to tolerable limits is made only one (0.8%) milk sample contained the quantity (263 ppb) over the limits of the Turkish Food Code (1). All the tetracyclin residues were found to be below the approved limits (1).

According to the results of different studies, in USA 75% (6) and 88% (9), in Canada 72% (9), in Europe 1.5% (27), in Portugal 30% (2) preservative additives have been found. The proportion had a ratio of 71% (6) and 28% (9) in terms of tetracyclin in USA. In a study performed in Turkey, it is reported that 11 (7.3%) of the 150 samples contained tetracyclin residue (10). In a study that was carried out in Poland, Streptomycin was found in 28 (11.1%) of 251 milk samples (26). Different contamination ratios in these studies can be explained by differences between analysis methods, regions and seasons at the time of sample collection.

In order to protect consumers from harmful effects of antibiotic residues, it's important to conform to waiting periods after treatment, search for preservative residues in raw milk brought to dairy plants and determine maximum limit values for any type of antibiotics. Producers who do not follow these guidelines must be faced with legal action.

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STAN ZAKAŻNYCH CHOROÓB ZWIERZĄT W POLSCE

według danych Głównego Inspektoratu Weterynarii w sierpniu 2004 r.*)

1. **BSE** – stwierdzono w województwie łódzkim (1-1) i podkarpackim (1-1).
2. **Wścieklizna zwierząt dzikich** – wystąpiła w 2 województwach: śląskim (1-2) i wielkopolskim (3-3). Zanotowano ją u 2 lisów, 1 jenota i 2 nietoperzy.
3. **Zgnielec amerykański pszczoł** – wystąpił w 4 województwach: mazowieckim (2-2), śląskim (2-2), warmińsko-mazurskim (1-1) i zachodniopomorskim (1-1).

*) W nawiasach podano liczbę powiatów i miejscowości, w których choroba została stwierdzona w okresie sprawozdawczym.