

USE OF SCREENING TESTS FOR CEFTIOFUR IN EVALUATION OF RAW MILK SAFETY

Dragan Ilievski

University “St. Kliment Ohridski” – Bitola, Faculty of Biotechnical Sciences – Bitola, RN Macedonia,
drdragan@t.mk

Biljana Trajkovska

University “St. Kliment Ohridski” – Bitola, Faculty of Biotechnical Sciences – Bitola, RN Macedonia,
biljana.trajkovska@uklo.edu.mk

Ljupche Kochoski

University “St. Kliment Ohridski” – Bitola, Faculty of Biotechnical Sciences – Bitola, RN Macedonia,
ljupce.kocoski@uklo.edu.mk

Abstract: In a modern dairy farming, it is getting more and more important cows to be treated with antibiotics with as less as possible withdrawal period for milk. Ceftiofur is one of such antibiotics that are recently widely used for cow's treatment against different diseases including mastitis. That's why a new generation of screening test kits sensitive for ceftiofur, like Milk Safe 3BTS, are available on the market. The aim of the study was to compare the results from different screening test on sensitivity of ceftiofur. In total 76 raw milk samples from sixteen treated cows were used during this study. Individual milk samples were tested using three commercial screening test kits for inhibitor residues: Milk Safe 3BTS, 4 Sensor and Eclipse Farm 3G. Results obtained during this study show different results. Milk Safe 3BTS gives the most positive results and higher sensitivity compare with the others. This is mainly due to different detection limits and only Milk Safe 3BTS has a specific sensitivity on ceftiofur residues. Generally, there are still residues of ceftiofur in milk sometimes even after 48 hours after application. Milk Safe 3BTS shows the better sensitivity results compares with the others. Milk Safe 3BTS can be used on a farm level as a screening test for detection of residues of ceftiofur.

Keywords: ceftiofur, antibiotics, milk, screening test, MRL

1. INTRODUCTION

Ceftiofur is a third generation cephalosporin antibiotic, used in veterinary medicine practice as a medication mostly for bacterial infections of the respiratory tract. It is used as hydrochloride in a dose up to 2 mg/kg BW/day up to 5 days, in lactating cows among all other animals. After injection, ceftiofur is rapidly metabolized in desfurloylceftiofur (DFC) and it is mainly excreted in urine and faeces, just only 0.15% of ceftiofur and DFC is excreted into the milk. Moreover, in healthy adult cows, ceftiofur metabolites are mostly (>90%) serum protein bound. This increases the excretion half-life (T_{1/2}) compared with most other cephalosporins and decreases the rate of excretion in milk. For therapeutic dosages lower than 2.2mg/kgBW in cattle, the residual concentration of DFC residues in cow milk is below the MRL. The recommended withdrawal period for lactating animals is nil; the milk of the treated animal may be sold for human consumption, provided that the milk composition also meets all the other quality criteria (L. Durel at al. 2019). It is clear that during milking, milk from treated animal will be mixed with milk from all other healthy untreated animals, so there is lower degree of chemical risk for the bulk milk in risk assessment process. Especially if milk from treated animal is mixed with nine parts of ceftiofur free milk, all the screening tests will give ceftiofur negative results (L. Durel at al. 2019).

The MRLs (maximum residue limits) for ceftiofur in milk is 100 µg/kg or ppb (EMA/MRL/835/02-FINAL, April 2002). The Committee for Medicinal Products within this Summary report 3 has amended Annex 1 in Council Regulation EEC (2377/90), allowing ceftiofur for intramammary use keeping the same MRL for milk in bovine species. According producer's information, it has 0 days withdrawal period for milk, so suddenly it has become first choice of vet doctors, but also special request from the dairy farmers, on the field in animal treatment, especially for all types of mastitis. This trend in the latest years has caused massive use of ceftiofur in farms all around North Macedonia, with serious participation of ceftiofur in usage of antibiotics in total. That trend has also caused many unclear situations in dairies in the country, having a lot of doubtful or false positive tests on commercial test kits, without any logical explanation. Finally, only few years ago, new test kit for ceftiofur was appeared on the market, MilkSafe™3BTS antibiotic test by Christian Hansen. In this way dairy producers can measure the residues of ceftiofur in milk samples from treated animals.

2. THE AIM OF THE STUDY

The aim of the study was to compare the results from different screening test on sensitivity of ceftiofur.

3. MATERIAL AND METHODS

Sixteen Holstein Frisian cows from one farm, different age and different stage of lactation were sampled by direct milking into sterile containers of 150 ml immediately before ceftiofur application and respectively 6, 12, 24, 48 and 72 hours after application until negative result. In total 76 samples were tested. The collected samples were analyzed on the same day. Lactating dairy cows were treated with ceftiofur (50 mg/mL), by intramuscular injection of one consecutive daily dose of about 2mg/kg BW. Raw milk was tested on ceftiofur with MilkSafe™3BTS antibiotic test from the producer Christian Hansen, 4 Sensor from the producer Unisensor and Eclipse Farm 3G from the producer Zeulab.

MilkSafe™3BTS antibiotic test is the newest screening test kit for rapid detection of antibiotics: β -lactam, tetracycline, ceftiofur and streptomycin. So far, this is the only test with specific sensitivity for ceftiofur. According information on producers website, detection limit on ceftiofur is 35 ppb (MilkSafeT 3BTS product insert)

4 Sensor is antibiotic test kit for detection of 4 group antibiotics: β -lactam antibiotics, tetracycline, chloramphenicol and streptomycin. Since ceftiofur belongs to wider β -lactam group of antibiotic, this test was used as a parallel test kit. According producer's website, test sensitivity on ceftiofur is 10-15 ppb (4sensor-bsct-kit060.pdf unisensor.be).

Eclipse Farm 3G is unspecific test for detection of antibiotics in milk, but also for all other inhibitors in milk. Since ceftiofur is still antibiotic, so by that is also inhibitor, this test was used as confirmation. It contains spores of *Geobacillus stearothermophilus* and pH indicator. Eclipse Farm 3G detection limits on ceftiofur is 50 ppb (Mata L. at al, 2016). Also, Navratilova P et al., (2020), in her study well stated that microbiological plate method using *Geobacillus stearothermophilus* showed the highest sensitivity in cephalosporin antibiotics, so that was the reason why we have used test based on this microorganism as eventual confirmation for other two screening tests.

4. RESULTS AND DISCUSSIONS

Results of the screening tests are presented in table 1. Results are presented as negative (- no reaction), positive (+ positive reaction). Only results for the first 6 hours after the injection are presented because all results before the application of ceftiofur are negative. From the results presented in table 1, we can see that there are still have positive milk samples on ceftiofur even 48 and 72 hours after application.

Table 1 Assessment of residues of ceftiofur and its metabolites in milk samples of sixteen dairy cows

Cow	after 6 hours			after 12 hours			after 24 hours			after 48 hours			after 72 hours		
	MS 3BTS	4 Sensor	Eclips Farm 3G	MS 3BTS	4 Sensor	Eclips Farm 3G	MS 3BTS	4 Sensor	Eclips Farm 3G	MS 3BTS	4 Sensor	Eclips Farm 3G	MS 3BTS	4 Sensor	Eclips Farm 3G
1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
2	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
3	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
4	-	-	-	-	-	-									
5	+	-	-	-	-	-									
6	+	+	-	+	+	-	+	+	-	-	-	-			
7	+	+	-	+	-	-	+	-	-	-	-	-			
8	+	+	-	+	+	-	+	+	-	-	-	-			
9	+	-	-	+	-	-	+	-	-	-	-	-			
10	+	+	+	+	+	-	+	+	-	+	-	-	-	-	-
11	+	+	-	+	-	-	+	-	-	-	-	-			
12	+	-	-	+	-	-	+	-	-	-	-	-			
13	+	+	+	+	+	-	-	-	-						
14	+	+	+	-	-	-	-	-	-						
15	+	-	-	+	-	-	-	-	-						
16	+	-	-	-	-	-	-	-	-						

The most sensitive test is MilkSafe3BTS on residues of ceftiofur in milk where we can detect lower MRLs of ceftiofur in milk. Similar results were presented by Durel L., et al., (2019) where they found that milk produced by individual cows dosed with 1 mg/kg BW ceftiofur HCL for five consecutive days can occasionally display positive or doubtful results to some of the screening tests commonly used in the dairy industry like Delvotest, ROSA test and SNAP test. On the other hand, Lim, J. H., et al., (2004) in his paper found lower concentration of ceftiofur than the proposed MRL (100 ng/mL) measured by liquid chromatography. According to, Jaglan, P. S., et al., (1992) the concentration of ceftiofur administered by intramuscular injection of 1.1 mg of ceftiofur/kg BW showed about 80 ng/mL at 12 h post-treatment. This value is in agreement with the present study where we noticed also residues of ceftiofur in milk samples after 48 hours post treatment. Also, higher sensitivity with MilkSafe3BTS was noticed with more positive samples compared with the other methods.

Ceftiofur, known as a “discard-zero” antibiotic, showed a positive result 72 hours after application in our study (table 2). The drug is marketed as an antibiotic that does not require a withdrawal period for lactating cattle. Results similar to this research for the presence of ceftiofur in milk were observed by Cristina et al. (2010) within a period of up to 12 h after ceftiofur antibiotic application. On the other hand Alves, J. F., et al., (2019) observed a positive reaction on ceftiofur 24 hours after application. However, various factors in unhealthy cows could modify drug behavior (Zonca, A., et al., 2011). Han, R., et al., (2017) reported that cow quarters with high- and mid-production rates eliminate the ceftiofur residues more quickly after the last intramammary treatment than cow quarters with low production do, both in healthy and infected cows. This means that drug elimination rates are influenced by the level of milk production (McKellar, Q. A., et al., 2004). These differences may due as a result on different screening test used in their study, parity, age of the cow and milk production.

Table 2 Assessment of residues of ceftiofur and its metabolites in milk samples

Number of cows	cow age (years)	parity	month of lactation	total absence of ceftiofur in milk after application (hours)
1	3.5	2	11	after 72 hours
2	3.5	1	10	after 72 hours
3	5.5	3	10	after 72 hours
4	4.5	2	6	after 12 hours
5	4	2	9	after 12 hours
6	3.5	1	2	after 48 hours
7	2.5	1	3	after 48 hours
8	10.5	5	4	after 48 hours
9	5.5	3	3	after 48 hours
10	3	1	2	after 72 hours
11	6	4	7	after 48 hours
12	5	3	5	after 48 hours
13	3.5	2	1	after 24 hours
14	5	2	1	after 24 hours
15	7	4	10	after 24 hours
16	7	3	2	after 24 hours

5. CONCLUSIONS

If ceftiofur is administered properly, it's residues are not likely to exceed MRL of 100 ppb. Milk Safe 3BTS is the only screening test specifically sensitive on ceftiofur, it is valuable on farm level as a control after cow treatment

with ceftiofur, preventing milk with even lower residues than MRL to reach dairy processors and be potentially harmful for human's health. A wider study should be performed in order to connect cow's age, lactation stadium and milk production to ceftiofur excretion rate through milk.

LITERATURE

- Alves, J. F., Paula, G. H., Silva, R. C., Leão, P. V., Leão, K. M., Nicolau, E. S., & Silva, M. A. (2019). Residues of antibiotics in milk: persistence and quality interference. *Canadian Journal of Animal Science*, 100(1), 93-101.
- Cristina, M., Melo, R.T., Mendonça, E.P., Coelho, L.R., & Rossi, D.A. (2010). Use of ceftiofur in dairy cows and their excretion in milk. *PUBVET*. 4: 931–937.
- Durel, L., Gallina, G., & Pellet, T. (2019). Assessment of ceftiofur residues in cow milk using commercial screening test kits. *Veterinary Record Open* 2019;0:e000329. doi:10.1136/ vetreco-2018-000329
- European Agency for the Evaluation of Medicinal Products, Committee for Veterinary Medicinal Products, Ceftiofur, Modification of MRLs for bovine species, Summary report 3, EMEA/MRL/835/02-FINAL, April 2002
- Fejzic, N., Begagic, M., Šerić-Haračić, S., et al. (2014). Beta lactam antibiotics residues in cow's milk: comparison of efficacy of three screening tests used in Bosnia and Herzegovina. *Bosn J Basic Med Sci* 2014;14: 155–9 <http://www.ncbi.nlm.nih.gov/pubmed/25172975>. doi: <https://doi.org/10.17305/bjbms.2014.3.109>
- Han, R., Li, S., Wang, J., Yu, Z., Wang, J., & Zheng, N. (2017). Elimination kinetics of ceftiofur hydrochloride in milk after an 8-day extended intramammary administration in healthy and infected cows. *PloS one*, 12(11), e0187261. 3455-3461.
- Jaglan, P. S., Yein, F. S., Hornish, R. E., Cox, B. L., Arnold, T. S., Roof, R. D., & Gilbertson, T. J. (1992). Depletion of intramuscularly injected ceftiofur from the milk of dairy cattle. *Journal of dairy science*, 75(7), 1870-1876.
- Lim, J. H., Park, B. K., Kim, M. S., Jang, B. S., Kim, D., & Yun, H. I. (2004). Residual determination of Ceftiofur in Raw Bovine Milk by Liquid Chromatography-Electrospray Mass Spectrometry. *Korean Journal of Veterinary Research*, 44(3), 367-371.
- Mata, L., Sanz, D., & Razquín, P. (2016). Performance of Eclipse Farm Test Coupled to e-Reader for Antibiotic Residues Detection in Raw Milk DOI:[10.1007/s12161-015-0210-y](https://doi.org/10.1007/s12161-015-0210-y); Corpus ID: 55682048 Published 1 February 2016 in Chemistry, Food Analytical Methods
- McKellar, Q. A., Sanchez Bruni, S. F., & Jones, D. G. (2004). Pharmacokinetic/pharmacodynamic relationships of antimicrobial drugs used in veterinary medicine. *Journal of veterinary pharmacology and therapeutics*, 27(6), 503-514.
- Navrátilová P., Vyhnálková, J., Zachovalová H., & Vorlová L. (n.d). *ACTA VET. BRNO* 2020, 89: 201–208; A microbiological multi-plate method to detect cephalosporin residues in milk, ; <https://doi.org/10.2754/avb202089020201>
- Schermerhorn P.G., Chu, P.-S., & Maureen, A. N. (1998). Determination of Cephapirin and Ceftiofur Residues in Bovine Milk by Liquid Chromatography with Ultraviolet Detection *JOURNAL OF AOAC INTERNATIONAL* VOL. 81, No. 5, 1998
- Zonca, A., Gallo, M., Locatelli, C., Carli, S., Moroni, P., Villa, R., & Cagnardi, P. (2011). Cefquinome sulfate behavior after intramammary administration in healthy and infected cows. *Journal of Dairy Science*, 94(7)