Scientific Research of the Union of Scientists in Bulgaria – Plovdiv,
series B. Natural Sciences and Humanities, Vol. XIII, ISSN 1311-9192,
International Conference of Young Scientists, 23 – 24 June 2011, Plovdiv

DETECTION OF β-LACTAM ANTIBIOTIC RESIDUES IN RAW AND COMERCIAL MILK

Velina Stefanovska[1], Vesna K. Hristova[1], Biljana Trajkovska[1], Nikolina Srbakoska[1], Julijana Tomovska[1], Stefce Presilski[1]
Velina Stefanovska[1] e-mail:velina85@hotmail.com
University St. Kliment Ohridski – Bitola
Faculty of biotehnical science

Abstract: *The present study was conducted to evaluate the extent of β-lactam antibiotic residues in unprocessed raw and comercial milk. Milk samples were randomly collected from farms in Pelagonia Region and markets. Charm II Luminometar and Hight Performance Liquid Chromatography (HPLC) methods were used to detect, identify and quantify the β-lactam antibiotic residues in milk. A total of 35 milk samples were screened. Among these 62,85% were negative and 37,15% positive for antibiotic residues. The zones size of positive samples appeared between 5,0 and 15,0 mm (mean 8,91±0,36mm). Residues level quantified between 0,4 to 400μg/L for Penicillin G, between 10 to 190μg/L for Amoxicillin, between 0,5 to 141μg/L for Ampicillin and between 2,1 to 122μg/L (40,74±10,59μg/L) for unknown antibiotics. The residues of Penicillium G (mean 59,53μg/L) in unprocessed milk was 14,9 and 11,9 fold, Amoxicillin (mean 36,11μg/L) 9,03 and 3,61 fold, Ampicillin (mean 46,91μg/L) 11,73 and 4,69 fold higher than (MRL's) standards of EU (4μg/L) and FDA (5-10μg/L), respectively.*

Key words: Milk, β-lactam, antibiotics, Penicillin residues.

Introduction: Antibiotics are vital medicines considered as the ultimate strategy to treat human infections. Their effectiveness is however, threatened by extensive and inapprppriate use od these, not only in medicine but also in agriculture. In veterinary practice, antibiotics are utilized at therapeutic levels primarily to treat diseases and to prevent infection. They are also used at subtherapeutic levels to increase feed efficiency, promote growth and prevent diseases.

The frequent use of antibiotics may result in drug residues that can be found at different concentration levels in products from animal origin, such as milk or meat. Presence of drugs or antibiotics residues in food above the maximum level recognized world wide by various public authorities is illegal (Kempe and Verachtert, 2000). Consumers want to be confident that their food supply is free of contamination by herbicides, pesticides, drugs or antibiotics due to the fact that they may cause potential health hazards, for example allergic reaction, carcinogenicity and promotion of the spread of bacterial resistance to antibiotics used in human medicines. Approximately 5-10 percent of the population is hypersensitive to Penicillin at a concentration as low as 1ppb or other antibiotics and suffers allergic reactions (skin rashes, hives, asthma, anaphylactic shock). Beside this, antibiotics may interfere with the manufacture of several dairy products. Concentra-

tion of 1 ppb delays starter activity during butter and yoghurt making. Antibiotics also decrease the acid anf flavor production associated with butter manufacture and they reduce the curdling of milk and cause improper ripening of cheese (Jones, 1999).

β-lactam is the oldest group of antibiotics which are frequently used for the treatment of sick animals in Macedonia. Milk of such animals is used without any safety measures, causing problems in dairy industries as well as in human health. However, the molecules belonging to the group of β-lactam have the lowest tolerance in the EU between all the antimicrobials. Consequently the EU regulations 2377/90 set the maximum residues limit (MRL) for some β-lactam antibiotics in milk for example, Penicillin G 4μg/L, Ampicillin 4μg/L, Dicloxacillin 30μg/L, Cephalexin 100μg/L and Cepharin 60μg/L (Ghidini et al.2002). Since, no work has been reported on antibiotic residues in raw and commercial milk. Thus, present study has been planned to evaluate the level of β-lactam antibiotics in row and commercial milk.

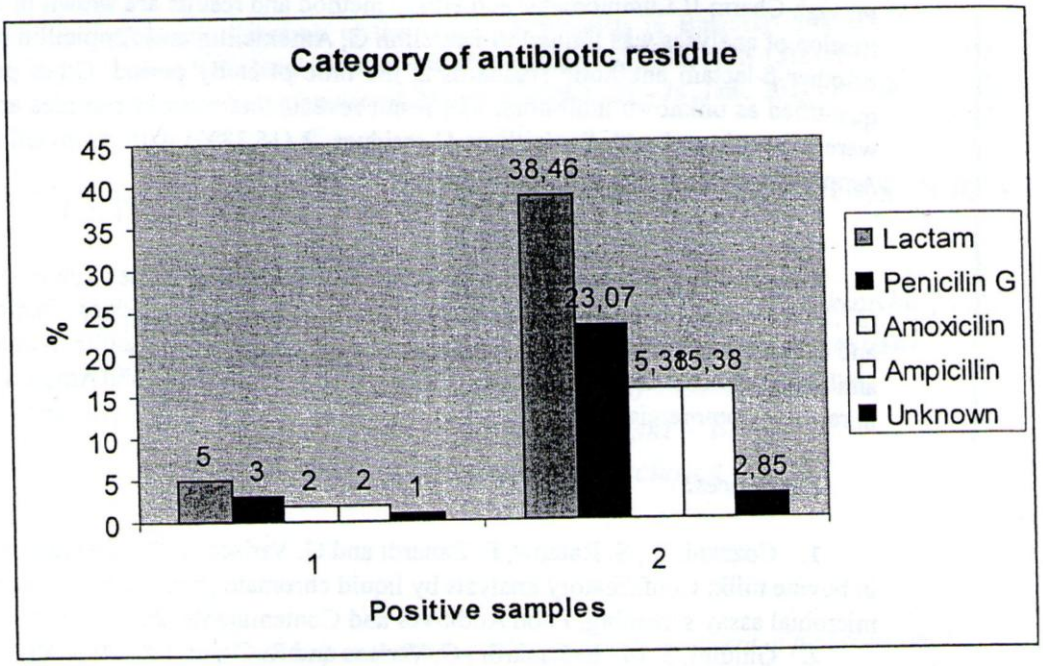
Matherials and Methods: Screening of milk samples for the presence of β-lactam antibiotic residues: A total od 35 unprocessed commercial and row milk samples from farms in Pelagonia Region and markets were collected and brought to the laboratory in Institute for health protection. Bitola, R. Macedonia. Milk samples were screened for the presence of β-lactam antibiotic residues using Charm II β-lactam test and HPLC method.

Quantification of β-lactam antibiotic residues: β-lactam antibiotic residues of row and commercial milk were quantified by the method as described by ghidni et al. (2002). A Hight Performance Liquid Chromatography (HPLC) system was used, which equipped with an autosampler (Model, L-2200), a Uv detector (Model, L-2400), a pump (Model, L-2130) isocratic, Column Oven (Model, L-2300) and C18 (ODS-3) column. The column effluent was monitored at a detector wave length of 365 nm.

Analyzes wich are presented in this study are made in the Institute for health protection, Bitola, R. Macedonia. Results are presented in table 1.

Tab 1: HPLC analysis of unprocessed row and commercial milk samples

Category of antibiotic residues	Positive samples (No.)	%
β-lactam residues	5	38,46
Penicillin G	3	23,07
Amoxicillin	2	15,38
Ampicillin	2	15,38
Unknown	1	2,85



Preparation of sample:

1. Charm II β-lactam test: green pill was taken in empty test tube, than 300±100µl water and 5,0±0,25 ml of sample or standard, mixed in vortex 10 times, incubated at 65±2°C for 2 minutes. Than test tube was removed from the incubator and yellow tablet was taken, after what was incubated at 65±2°C for 2 minutes, centrifuged 3 minutes, 300±100µl water and scintilacic liquid was added, test tube was closed and mixed in vortex.

2. HPLC method: Milk sample (5ml) was taken in 10ml sterilized pyrex screw cap centrifuge tubes and vortex mixed with 10% aqueous solution of acetic acid (400µl). The acidified milk was taken centrifuged at 3500 rpm for 10 minutes at 4°C in the Backman centrifuge machine. The clear supematant phase was taken layer and filtered through a 4,05µm nylon filter (13mm diameter) with the back pressure of pump. The filtered extract was put into 2ml sterilized vials and injected (5µl) into HPLC system. Each sample was preparated in duplicate.

Preparation of standard: Stock standard solutions at a concentration of 100µg/ml of Ampicillin (Sigma), Penicillin G (Sigma) and Amoxicillin (Sigma) were preparated individually in deionized water, previously filtred in 0,45µm filter paper. Mixed standard working solution containing Ampicillin, Amoxicillin and Penicillin G were preparated from the stock solution at concentrations level of 0.2µg/ml, 0.4µg/ml, 0.6µg/ml, 0.8µg/ml, 1.6µg/ml, 2.0µg/ml, 2.4µg/ml, 3.0µg/ml and 4.0µg/ml. the solutions were stored at freezing temperature till use (not more than one month).

Quantifications: The quantifications of analysis was carried out by injecting standard solution, blank sample and spiked samples. For each β-lactam analyzed, the factor of response consisting of the ratio between the height of analyte peak and height of internal standard was verified.

Results and Discussions: Screening of milk samples for β-lactam antibiotic residues: Raw and commercial (unprocessed) milk samples were screened for β-lactam antibiotic residues

through Charm II Luminometar and HPLC method and results are shown in Table 1. The quantification of analyses was limited to Penicillin G, Amoxicillin and Ampicillin due to unavailability of other β-lactam antibiotic standards at the time of study period. Other peaks appeared were quantified as unknown inhibitors. The result reveals that over 13 samples analyzed, 3 (23,07%) were contaminated with Penicillium G residues, 2 (15,38%) with Amoxicillin, 2 (15,38%) with Ampicillin and 1 (2,85%) with unknown inhibitors.

Conclusions: Among 35 raw and commercial milk samples (unprocessed) screened, 13 (37,15) concluded to be contaminated with β-lactam antibiotic residues . Penicillin residues in raw and commercial milk samples were dominant followed by Amoxicillin, Ampicillin and unknown antibiotics. Remarkably higher level of Penicillin G, Amoxicillin and Ampicillin residues revealed in raw and commercial milk contrast to Maximum residual Limits (EU and FDA standard).

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Reviewer: Prof. J. Simov, PhD