Dietary exposure assessment of ß-lactam antibiotic residues in milk on Croatian market

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professional paper

Summary

This paper presents the preliminary screening report of the occurrence of β-lactam antibiotic residues in milk on Croatian market. The intensive animal production sometimes leads to the unavoidable presence of residues of veterinary drugs in food. However, it is possible to reduce the presence of residues in foods of animal origin by using the principles of good veterinary and good manufacturing practices, continuous control of food and using risk assessment approach. Method used for determination of these antibiotics were validated according to the guidelines laid down by European Commission Decision 2002/657/EC. The estimated dietary exposure based on the data on average consumption of milk and the estimated concentration of amoxicillin, ampicillin, benzylpenicillin, cloxacillin, cephapirin, cefazolin, cefoperazone and ceftiofur does not exceed relevant toxicological reference value (acceptable daily intake). These indicate that milk on Croatian market contain very low levels of β-lactam antibiotic residues and toxsicological risk with regard to consuming of milk could not be considered as a public health problem.

Keywords: dietary exposure, β-lactam antibiotics, milk, food safety

Introduction

Among antimicrobial drugs that have been used in animal production more than five decades, β-lactam antibiotics probably are the most widely used class of drugs in veterinary medicine. The frequent use of antibiotics may result in drug residues that can be found at different concentration levels in products from animal origin, especially in milk (Gustavsson and Sternesjö, 2004; Hussein et al., 2005; Kožarová et al., 2004; Popelka et al., 2005; Vragović et al., 2011). In recent years there has been much debate over the use of antimicrobials in livestock production, and their potential to create resistant bacteria and transfer of such genes to bacteria that are important to human population (Hamscher et al., 2002; Lathers, 2001; Teuber, 2001; WHO, 2001; Witte, 2000). As a consequence of frequent use, violative levels of β-lactam residues occasionally occur in the milk, constituting potential problems not only to the consumer (Hamscher et al., 2002; Schwarz and Chaslus-Dancla, 2001; Teuber, 2001; Vragović et al., 2011; Witte, 2000) but also to the production of dairy products, like decrease the acid and flavor of butter and they reduce the curdling of milk and cause improper ripening of cheese (Brady and Katz, 1988; Currie et al., 1998; Dewdney et al., 1991; Hadžiosmanović et al., 2003; Jones, 1999; Mourot and Loussourorn, 1981). Risk assessment can help in understanding the connection between reducing the risks that may be associated with food and reducing the risk to consumers from the harmful health effects, so this approach is of particular importance in the development of appropriate food safety control (Hathaway, 2003; JECFA, 2005; Kroes, 2005; Vragović et al., 2009).
The paper presents the results of first screening dietary exposure assessment in Croatia for the general population associated with the β-lactam antibiotic residues in consumed milk.

Materials and Methods

Sampling and chemical analysis

We analyzed 105 samples of milk on the content of β-lactam antibiotics. The samples are collected through a year period (2010) from monitoring and surveillance programs on a random basis. Samples were stored at +4 °C until analysis. For further investigations, samples were stored at -22 °C for a month.
Receptor-based screnning method Twinsensor^{BT} Milk test® was used for determination of β-lactam. The test

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procedure was carried out exactly as per test kit instructions. The milk sample (200 µl) was applied into the microwell, mixed with the reagents and incubated for 3 minutes. The dipstick was dipped into each of the microwell, laid in the incubator and incubated for 3 more minutes. Dipsticks were visually verified that strip has a valid development: central control line should be visible. The test was fully validated, not only in our laboratory which is accredited according to EN ISO/IEC 17025, but also through international ring trial validation study (Ooghe and Reybroeck, 2006). According to Council Directive 2002/657/EC (EC, 2002), only methods that do not exceed 5 % of false negative results at MRL values may be used for screening purposes. Our validation included determination of detection capability ($CC\beta$), specifity and the percentage of false negative and false positive results. The blank milk samples (n = 20) were tested all as negative, i.e. the false positive rate of Twinsensor ^{BT} test was determined as zero percent. All blank samples spiked with ß-lactams at the declared sensitivity (amoxicillin 3-6 µg/l; ampicillin 3-5 µg/l; benzylpenicillin 2-3 µg/l; cloxacillin 4-8 µg/l; nafcillin 40-50 µg/l; cephapirin 4-8 µg/l; cefazolin 20-25 µg/l; cefoperazone 2-3 µg/l and ceftiofur 10-15 µg/l) were detected correctly as positive, i.e. at these concentrations there were no false negative results. Twinsensor^{BT} test is not suitable for the detection of nafcillin in milk samples because the detection capability is much greater than the regulatory maximum residue limit (*MRL*), and therefore nafcillin was not subject of estimated exposure assessment. Detection capability or limit of detection (*LOD*) for other ß-lactams are acceptable. Because the manufacturer gave a limit of detection at not fixed concentration, and sensitivity can vary from batch to batch, in this study we carried validation at the next concentration of ß-lactams in milk (n = 20): amoxicillin and ampicillin at 3 µg/l; benzylpenicillin at 2 µg/l; cloxacillin at 4 µg/l, cephapirin at 5 µg/l, cefazolin at 20 µg/l, cefoperazone at 2 µg/l, ceftiofur at 10 µg/l and nafcillin at 60 µg/l.

Milk consumption data

Estimation of consumption for milk was carried out based on surveys of the Croatian Central Bureau of Statistics (*CBS*) and the data on consumption used by Joint FAO/WHO Expert Committee on Food Additives – *JECFA* (CBS, 2010; JECFA, 2005, 2006).

The Survey in Croatia is in line with the Eurostat methodological recommendations and international standards and classifications and covers only private households, which means that population residing in institutions, boarding schools, prisons, hospitals etc. is excluded. The Survey is conducted as an annual survey and data are collected continuously in the course of a year in 26 two-week intervals. Every other week, a part of the total sampled households are interviewed. The sample frame used for the selection of dwellings occupied by private households was based on the 2001 Census of Population, Households and Dwellings. Thus, 4 670 dwellings occupied by private households were selected for sample. At each selected occupied dwelling, all private households were interviewed. There were 3 004 private households successfully interviewed (CBS, 2010). Data show that average consumption of milk in the Republic of Croatia is 0.222 kg/person/day, while consumption which is evaluated by *JECFA* is a much higher (1.5 kg/person/day).

Calculation of estimated dietary exposure

Daily intake are estimated using the formula in which chemical concentration refer to amount of chemical in food, consumption refer to the amount of food consumed and dietary exposure refer to the amount of chemical ingested via food.

Dietary exposure [µg/kg] = Σ (Food chemical concentration [µg/kg] × Food consumption [kg/person])

Results and Discussion

This is the first report of the occurrence of β-lactam antibiotic residues in milk on Croatian market and none of the analyzed samples of milk showed that it contains the residues of ß-lactam antibiotics above the *LOD*. In a risk assessment, the handling of ‘‘non-detects’’ residues can be crucial, especially when most of the samples are ‘non-detects’ (JECFA, 2005). In our experiment proportion of results below *LOD* was more than 80 % and we took in calculation all results below *LOD* as samples that containing antibiotics less than *LOD*1.6 (GEMS/Food, 1995). Based on that amount of the β-lactam antibiotics and data on average consumption of milk in the Republic of Croatia and data on consumption which is evaluated by *JECFA* (CBS, 2010; JECFA, 2006) we performed the estimated daily intake and results are stated in Table 1.

Table 1. Combined data for estimated daily intake of ß-lactam antibiotic residues in milk

Compound	Estimated concentration in milk* [µg/kg]	EDI[1] based on CBS[2] consumption [µg/person]	Level of achievement ADI[3] [%]	EDI[1] based on JECFA[4] consumption [µg/person]	Level of achievement ADI[3] [%]	ADI[3] [µg/kg body weight]	MRL [µg/kg]
Amoxicillin	4.8	1.06	0.009**	7.2	0.06**	0-200**	4
Ampicillin	4.8	1.06	3.55***	7.2	24***	0-0.5***	4
Benzylpenicillin	3.2	0.71	2.37	4.8	16	0-0.5	4
Cloxacillin	6.4	1.42	0.012**	9.6	0.08**	0-200**	30
Cephapirin	8.0	1.78	0.148**	12	1**	0-20**	60
Cefazolin	32	7.11	0.119	48	0.8	0-100	50
Cefoperazone	3.2	0.71	0.024****	4.8	0.16****	0-50****	50
Ceftiofur	16	3.55	0.119	24	0.8	0-50	100

1. Estimated daily intake (EDI); 2. Central Bureau of Statistics (CBS); 3. Acceptable daily intake (ADI) 4. Joint FAO/WHO Expert Committee on Food Additives (JECFA); *value of LOD 1.6: **value of ADI according to OCS/OHP Australija; ***according to ADI for benzylpenicillin; **** according to ADI for ceftiofur

According to *CBS* data on average consumption of milk and the estimated concentration of β-lactam residues, the estimated daily intake of amoxicillin, cloxacillin, cephapirin, cefazolin, cefoperazone and ceftiofur is assessed < 1 % of acceptable daily intake (*ADI*), while the of ampicillin and benzylpenicillin is assessed 1 - 5 % of *ADI*.

Since the consumption of milk according to *JECFA* far greater, it is logical that the estimated intake, with the same concentrations of β-lactam antibiotics, is much higher. For milk consumption data according to *JECFA* the estimated daily intake for ampicillin and benzylpenicillin was assessed > 5 % of *ADI* but for amoxicillin, cloxacillin, cephapirin, cefazolin, cefoperazone and ceftiofur was assessed < 1 % of *ADI*.

For conducting exposure assessments should be established a framework that risk assessor will allow to select the most appropriate methodology for the intended use of the assessment. In general, the framework in early steps will include screening methods that use minimal resources and the shortest possible time may to identify, among the large number of chemicals, which are, or are not, safety concern. Certainly, further assessment is not needed for substances that do not present safety concerns when analysed using screening methods.

Conclusions

The choice of data on the concentration of chemicals in food depends on the desired level of public health protection. The method used for β-lactam antibiotics determination in milk was validated according to the Commission Decision 2002/657/CE and showed that was rapid and simple but, we are aware of deficiencies in the assessment using screening methods for determination of contaminants in food. However, this approach is acceptable because it allows a quick understanding of possible risks to human health as a result of taking food with residues of veterinary medicines; so on the basis of these preliminary findings can be undertaken extensive research and use more reliable methods for determining residues. Estimated exposure to β-lactam antibiotic residues in milk on Croatian market does not exceed relevant toxicological reference values (*ADI*) and therefore milk are safe for human consumption.

References

Brady, M.S., Katz, S.E. (1988): Antibiotic/antimicrobial residues in milk, *J. Food Prot*. 44, 8–11.

CBS (2010): Central Bureau of Statistics – Republic of Croatia, Statistical Yearbook, pp.194-200.

Currie, D., Lynas, L., Kennedy, G., Mc Caughey, J. (1998): Evaluation of modified EC four plate method to detect antimicrobial drugs. *Food Addit. Contam*. 5, 651–660.

Dewdney, J.M., Maes, L., Raynaud, J.P., Blanc, F., Scheid, J.P., Jackson, T., Lens, S., Verschueren, C. (1991): Risk assessment of antibiotic residues of β-lactams and macrolides in food products with regard to their immuno-allergic potential. *Food Chem. Toxicol*. 29, 477-483.

EC (2002): European Commission. Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.

GEMS/Food-Euro (1995): Second workshop on reliable evaluation of low-level contamination of food. Kulmbach, Federal Republic of Germany, 26–27 March.

Gustavsson, E., Sternesjo, A. (2004): Biosensor Analysis of betalactams in Milk: Comparison with Microbiological, Immunological, and Receptor-Based Screening Methods. *J. AOAC Int*. 87, 614-620.

Hadžiosmanović, M., Mioković, B., Njari, B., Kozačinski, L., Pranjić, D., Cvrtila, Ž. (2003): Remains of biologically active substances (Rezidua, Bio-Rezidua) in food of animal origin. *Meso* 5, 52-58.

Hamscher, G., Sczesny, S., Höper, H., Nau, H. (2002): Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. *Anal. Chem*. 74, 1509–1518.

Hathaway, S.C. (2003): Risk assessment procedures used by the Codex Alimentarius Commission and its subsidiary and advisory bodies. ALINORM 93/97. Codex Alimentarius Commission. Geneva.

Hussein, K., Marcinčák, S., Máté, D., Kožarová, I., Sokol, J., Zdolec, N. (2005): Use of Premi®Test for detection of sulphonamide residues in chicken eggs, *Acta Veterinaria* (Beograd) 55, 493-500.

JECFA (2005): Joint FAO/WHO Expert Committee on Food Additives. Dietary exposure assessment of chemicals in food, Annapolis, Maryland, USA.

JECFA (2006): Joint FAO/WHO Expert Committee on Food Additives. Updating the Principles and Methods of Risk Assessment: MRLs for Pesticides and Veterinary Drugs, Rome.

Jones, G.M. (1999): On farm test for drug residues in milk, Virginia cooperative extension, Knowledge for the common wealth, Virginia Polytechnic and State University, U.S.A.

Kožarová, I., Máté, D., Hussein, K., Raschmanova, K., Marcinčák, S., Jevinová, P. (2004): High-performance liquid chromatographic determination of sulfadimidine residues in eggs. *Acta Veterinaria* (Beograd), 54, 427-435.

Kroes, R., Kleiner, J., Renwick, A., 2005. The Threshold of Toxicological Concern Concept in Risk Assessment. *Toxicol. Sci.* 86, 226–230.

Lathers, C.M. (2001): Role of veterinary medicine in public health: antibiotic use in food animals and humans and the effect on evolution of antibacterial resistance. *J. Clin. Pharmacol*. 41, 595-599.

Mourot, D., Loussourorn, S. (1981): Sensitivities of lactic antibiotic used in veterinary medicine. *Rev. Méd. Vét.* 157, 175–177.

OCS/OHP (2008): ADI List, Acceptable daily intakes for Agricultural and Veterinary chemicals, Department of Health and Ageing Office of Chemical Safety, Australian Government.

Ooghe, S., Reybroeck, W. (2006): Reporting Ring Trial Twinsensor, T&V-ILVO Melle, Scientific Institute of Flemish Community.

Popelka, P., Nagy, J., Germuška, R., Marcinčák, S., Jevinová, P., De Rijk, S. (2005): Comparison of various assays used for detection of beta-lactam antibiotics in poultry meat. *Food Addi.Contam*. 22, 557-562.

Schwarz, S., Chaslus-Dancla, E. (2001): Use of antimicrobials in veterinary medicine and mechanisms of resistance. *Vet. Res.* 32, 201-225.

Teuber, M. (2001): Veterinary use and antibiotic resistance. *Curr. Opin. Microbiol*. 4, 493–499.

Vragović, N., Grubelić, M., Stuhne, G., Sapunar-Postružnik, J., Bažulić, D. (2009): Risk assessment of veterinary drug residues In abstracts of XXX3th. Conference of Croatian Nacional Institute of Public Health, Poreč, Croatia.

Vragović, N., Bažulić, D., Njari, B. (2011): Risk assessment of streptomycin and tetracycline residues in meat and milk on Croatian market. *Food Chem. Toxicol.* 49, 352-355.

Witte, W. (2000): Selective pressure by antibiotic use in livestock. *Int. J. Antimicro. Ag*. 16, 19–24.

WHO (2001): World Health Organization. Antimicrobial use in Livestock and the problem of bacterial resistance in human.

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