Analytical strategies for residue analysis of veterinary drugs in milk

Roca, M.[1], Althaus, R. L.[2], Borrás, M[1], Beltrán M. C.[1] & Molina M. P.[1]

[1]Instituto de Ciencia y Tecnología Animal. Universidad Politécnica. 46022 Valencia, Spain
[2]Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, 3080 Esperanza, Argentina

Cattle Production Free Communication
Session 38 Poster 20
Abstract 4079

Introduction

- Drug residues in milk may cause public health and technological problems
- A program of milk quality control and traceability has been èstablished in Spain (RD 1728/2007)
- Screening methods (microbiological, enzymatic, protein receptor-binding, etc.) are used to detect antibiotics in milk

Objective

The aim of this study was the evaluation of the most important screening methods and to establish analytical strategies to detect the most commonly antibiotics used in cow milk in Spain

Materials & Methods

Milk samples

Cow milk samples
- SAT MORE, CEATEL Valencia (Spain)

Milk analysis
- Composition, RCS, bacteriology LICOVAL (Valencia, Spain)

- ISO/FDIS 13969/FIL 183: 2002
- ISO/FDIS 18330/ FIL 188: 2002

Specificity: 100 negative milk samples/method
Sensitivity: 10 milk samples/MRL/antibiotic

Screening methods

Microbiological
- BRT-AiM
- Charm Blue Yellow
- Copan
- Delvotest Accelerator
- Delvotest SP-NT
- Eclipse 50 & 100

Specific
- Penzym
- Beta Star
- Delvo XP βL
- ROSA MRL (βL & TET)
- SNAP (βL & TET)
- Twinsensor

Antibiotics

Antibiotics	MRL (µg/Kg)	Use Frecuency (%)
Amoxycillin	4	4.57
Ampicillin	4	3.81
Cloxacyllin	30	4.95
Dicloxacillin	30	1.90
Penicillin	4	38.10
Cefalexin	100	3.81
Cefalonium	20	1.52
Cefoperzone	50	1.90
Cefquinome	20	2.29
Oxytetracycline	100	0.19
Neomycin	1500	12.57
Gentamycin	100	5.71
Erytromycin	40	1.14
Estreptomycin	200	6.48
Kanamycin	150	4.95
Lincomycin	150	1.52
Enrofloxacin	100	1.52
Colistin	50	3.05

Statistical analysis

- Statistical significant differences:
  - Specificity: (Negative results/ Total samples) x 100
    - ➡ Analysis of frequency: χ^2 test
  - Sensitivity MRL: (Positive results/Total samples) x 100
    - ➡ Logistic regression: $L_i = \text{logit}[P_i] = \beta_0 + \beta_1 [M] + \varepsilon_i$

logit [P_i] = probability of positive results of a method; β_0, β_1 = coefficients; M_i = method (M=7 for microbiological; M= 6 specific methods for β-Lactams and M=3 for tetracyclines) and ε_{ij} = residual error

- Comparison of methods: *Cluster* analysis
- Detection percentages through a combination of two methods (microbiological and specific)

Results

Specificity

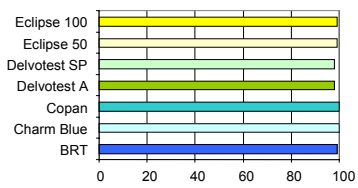

Figure1. Specificity (%) of microbiological methods

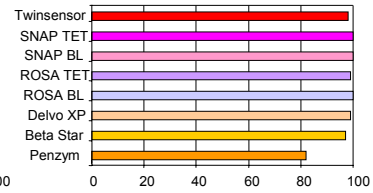

Figure 2. Specificity (%) of specific methods

Sensitivity

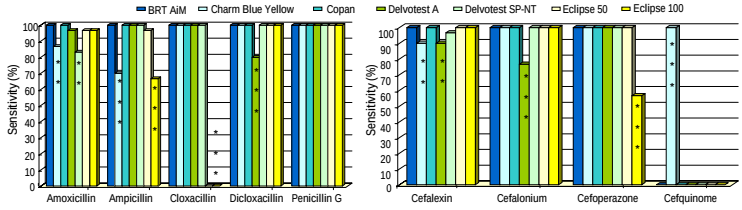

Figure 3. Sensitivity (%) at MRL of microbiological methods for β-Lactams

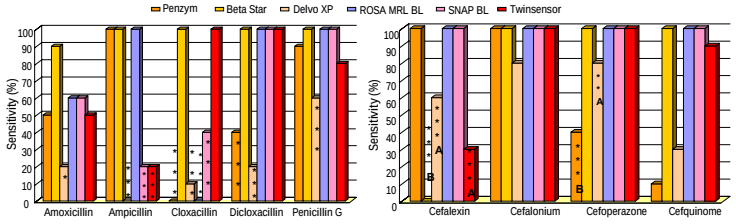

Figure 4. Sensitivity (%) at MRL of specific methods for β-Lactams

***: p<0.001; **: p<0.01: Statistical significant differences between methods for each antibiotic from the χ^2 test; A, B: Statistical significant differences between methods (p<0.01)

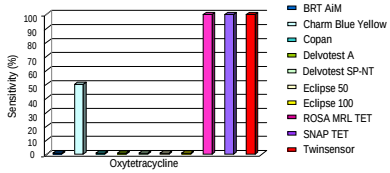

Figure 5. Sensitivity (%) at MRL of screening methods for oxytetracycline

Cluster analysis

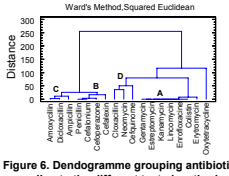

Figure 6. Dendogramme grouping antibiotics according to the different tested methods

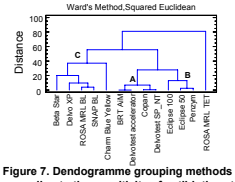

Figure 7. Dendogramme grouping methods according to the sensitivity of antibiotics at MRL

Table 1. Antibiotic detection pattern in a combination of microbiological and specific methods

Methods		Detection Percentage	Detection
Microbiological	Specific		
BRT-AiM	Twinsensor	76.55%	100 % Penicillins 100 % Cephalosporins 100% Oxytetracycline 100% Neomycin
Charm Blue Yellow	Beta Star	75.8 %	99.1 % Penicillins 96.0 % Cephalosporins 53.3 % Oxytetracycline 100 % Neomycin 100 % Erytromycin
Delvotest Accelerator	Twinsensor	74.85%	99.7 % Penicillins 93.6 % Cephalosporins 100 % Oxytetracycline 100 % Neomycin
Eclipse 100	Twinsensor	73.9%	97.3 % Penicillins 97.6 % Cephalosporins 100 % Oxytetracycline 100 % Neomycin

Conclusions

- Screening methods can not detect gentamycin, estreptomycin, kanamycin, lincomycin and colistin, used in 21.27% in mastitis treatment in Spain
- The combination of two methods (microbiological and specific) enables the detection of approximately 75% of the antibiotics used

➡ *There is no screening method available to detect all antimicrobials used for mastitis treatment in Spain. Therefore, the detection pattern of existing methods and/or development of new tests making a more precise and efficient analytical strategy possible becomes imperative*

Acknowledgment

This work forms part of the studies 51.07 and 29.08 supported by the Spanish Ministry of the Environment and Rural and Marine Affairs (Madrid, Spain). The authors also wish to thank the Polytechnic University of Valencia (Spain) for funding the collaboration of Dr. Rafael Althaus