PHOTOMETRIC DETECTION OF ANTIBIOTIC RESIDUES IN MILK BY A MICROBIOLOGICAL SYSTEM

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SUMMARY

The aim of this study was to determine the detection capabilities of the ResScreen microbiological system (BT and BS Bioassays) for 26 antimicrobial agents in milk by photometric measurement. In order to carry out this study 16 replicates of twelve concentrations using samples of milk from individual cows were tested. The detection capabilities, determined by means of logistic regression models for the BT and BS bioassays were, respectively (μg/l): amoxicillin (8, 5), ampicillin (8, 4), cloxacillin (42, 42), oxacillin (19, 17), penicillin G (4, 4), cefadroxil (120, 180), cephalexin (96, 150), cefoperazone (80, 120), ceftiofur (75, 90), cefuroxime (113, 140), clortetracycline (400, 3300), oxytetracycline (118, 630), tetracycline (180, 620), sulfadiazine (41200, 106), sulfamethazine (3300, 355), sulfamethoxazol (9800, 78), sulfathiazole (10700, 115), gentamycin (640), neomycin (1200), streptomycin (2600), erythromycin (350), lincomicin (350), tylosin (100), ciprofloxacin (2855), enrofloxacin (2000) and marbofloxacin (3900). The simultaneous use of both bioassays identifies betalactam, tetracycline and sulfamide residues in milk. Also, neomycin, tylosin and lincomycin residues can be detected, but their residues can be confused with betalactam antibiotics since these molecules produce positive results in BT and BS bioassays.

Key words: antimicrobial, detection capability, milk, microbial inhibitor test, photometric detection.

RESUMEN

Detección fotométrica de residuos de antibióticos en leche mediante un sistema microbiológico.

El objetivo de este trabajo ha sido determinar las capacidades de detección del sistema microbiológico ResScreen (Bioensayos BT y BS) sobre 26 agentes antimicrobianos empleando mediciones fotométricas. Para llevar a cabo este estudio, se ensayaron 16 replicas de doce concentraciones

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utilizando muestras de leche procedentes de vacas individuales. Las capacidades de detección de los bioensayos BT y BS, calculadas mediante el modelo de regresión logística, fueron las siguientes (µg/l): amoxicilina (8, 5), ampicilina (8, 4), cloxacilina (42, 42), oxacilina (19, 17), penicilina G (4, 4), cefadroxilo (120, 180), cefalexina (96, 150), cefoperazone (80, 120) ceftiofur (75, 90), cefuroxime (113, 140), clortetraciclina (400, 3300), oxitetraciclina (118, 630), tetraciclina (180, 620), sulfadiazina (41200, 106), sulfametazina (3300, 355), sulfametoxazol (9800, 78), sulfatiazol (10700, 115), gentamicina (640), neomicina (1200), estreptomicina (2600), eritromicina (250), lincomicina (250), tilosina (100), ciprofloxacina (2855), enrofloxacina (2000) y marbofloxacina (3900). El uso simultáneo de ambos bioensayos permite identificar residuos de antibióticos betalactámicos, tetraciclinas y sulfonamidas en la leche. También pueden detectarse residuos de neomicina, tilosina y lincomicina, pero debido a que estas moléculas producen resultados positivos a los bioensayos BT y BS, sus residuos pueden confundirse con antibióticos betalactámicos.

Palabras clave: antimicrobianos, capacidad de detección, leche, método de inhibición microbiológica, detección fotométrica.

INTRODUCTION

The presence of certain antibiotic residues in milk constitutes a potential risk for the consumer as they may cause allergic reactions as well as interference with intestinal flora and the development of resistance to antibiotics (Dewdney *et al.*, 1991; Currie *et al.*, 1998; Demoly & Romano, 2005; Wilke *et al.*, 2005). From a technological point of view, antibiotic residues can produce important losses in fermented products, such as cheese (Mourot & Loussourorn, 1981; Brady & Katz, 1987; Packham *et al.*, 2001; Berruga *et al.*, 2007; Bradley & Green, 2009). Therefore, monitoring antibiotic residues is very important in controlling food safety. For these reasons, the Codex Alimentarius Comission has established safe levels of antimicrobials in edible tissues (Codex Alimentarius, 2009), and the EU has regulated the Maximum Residue Limits (MRLs) allowed in milk and other animal foodstuff in the Council Regulation 37/2010/CE.

The European Union from EEC657/2002 Directive (Commission Decision, 2002) classifies the analytical methods for detection of inhibitory substances as qualitative or quantitative methods based on their characteristics and methodology. Thus, several commercially available qualitative tests have been developed for the swift and precise detection of the presence of antibiotic residues in milk (Diserens *et al.*, 2005; Toldra & Reig, 2006). Many of the screening tests are based on the inhibition of microorganism growth caused by the presence of drug residues. Among the most widely used microorganisms, *Geobacillus stearothermophilus* subsp. *calidolactis* has been employed in tests such as Delvotest (Kelly, 1982; IDF, 1991), BRT-AiM (IDF, 1991; Müller & Jones, 1993), Eclipse (Montero *et al.*, 2004), Charm AIM-96 (Zomer & Lieu, 1995) and "BT"-"BS" ResScreen (Nagel *et al.*, 2011).

The ResScreen® microbiological system uses two bioassays: "BT" (betalactams and tetracyclines) and "BS" (betalactams and sulfonamides) containing *Geobacillus stearothermophilus* subsp. *calidolactis*

spores, culture media, indicators (acid-base and redox) and specific synergistic components to identify betalactams, tetracyclines (Nagel *et al.*, 2009a) and sulfonamides (Nagel *et al.*, 2009b) residues in milk. This system complies with the International Standardization Organization guidelines of ISO / TS 26844-IDF/RM 215 (IDF, 2006).

In general, the microbiological methods are economical, easy to perform and can analyze a large number of milk samples. However, visual interpretation of the results can be subjective due to individual differences in each laboratory. In order to avoid subjective differences in the visual interpretations and read test results in an automated and more objective manner, some authors (Luitz & Suhren, 1995; Luitz *et al.*, 1996; Althaus *et al.*, 2003) propose performing photometric measurements by means of the microtiter plate reader. Consequently, the aim of this research was to calculate detection capabilities of the ResScreen® microbiological system for 26 antimicrobial agents in milk by means of a photometric reader in order to reduce errors due to subjective interpretations.

MATERIALS AND METHODS

1. Milk samples: The animals belonged to dairy herds of Las Colonias (Santa Fe, Argentina). For this study, milk samples corresponding to the morning machine milking session (6.00 am) of 16 cows were collected in the 60-90 day period postpartum. The animals received no pharmacological treatment throughout the sampling period (IDF, 2002).

The samples selected had a chemical composition and pH values considered as normal for bovine milk, low somatic cell counts (SCC < 400000 cells/ml), and a bacterial count acceptable for cow milk (CFU < 100000 cfu/ml).

2. Antimicrobial solutions and spiked samples: Drugs for preparation of the antimicrobial solutions were stored and handled according to the manufacturers' instructions before use. All the dilutions were prepared in 100 ml volumetric flasks at the time the analyses were carried out in order to avoid the possibility of unstable solutions. Table 1 summarizes the concentrations utilized for the analysis of the 26 antimicrobial agents.

The antimicrobial solutions were prepared in one step from the respective stock solution using antimicrobial-free milk (IDF, 2002) determined previously by the "BT" and "BS" bioassays. Final concentrations of drugs in milk (µg/l) were achieved after serial dilutions, in such a way that the volume of the antimicrobial agent solution did not exceed 1 % of the volume of the final solution to be analyzed. Thus, 12 concentrations were prepared with different levels of each drug. For each concentration, 16 replicates were prepared using antibiotic-free milk samples obtained from individual animals (IDF, 2002).

3. ResScreen® system: The ResScreen® microbiological system (ResScreen® S.R.L., Esperanza, Argentina) procedure for detection and identification of antibiotic residues in milk is based on inhibition of spore outgrowth of organisms such as *Geobacillus stearothermophilus* subsp. calidolactis C-953. Growth of the microorganisms is evidenced by the colour change of the acid-base ("BT" bioassay) or redox ("BS" bioassay) indicator present in both methods.

This system was carried out according to the manufacturer's instructions. Briefly, 50

µl milk sample were added to individual plates of the "BT" and "BS" bioassays. Plates were incubated in a water bath at 64 1°C for 3 ("BT" bioassay) and 4 ("BS" bioassay) hours until the colour change of the negative samples had taken place. Immediately after the incubation period, absorbance was measured with a Microplate reader (Biotek ELx800™, Biotek Instrument Inc., Winooski, Vernont, USA) using a wavelength of 550 nm. The photometric measurements were expressed in relative absorbances, according to the following transformation:

$$\%A = (A_x - A_0)/(A_{100} - A_0)\ 100$$

Where: %A: relative absorbance, Ax: milk sample absorbance with "x" antibiotic concentration, A_0: absorbance of antibiotic free milk (negative control), A_{100}: milk sample absorbance with antibiotic that produces 100% of positive results.

4. ***Detection capability*** **($CC\beta$)**: The ResScreen® system ("BT" and "BS" bioassays) detection capabilities of the antimicrobial agents were established in line with the IDF indications (IDF, 2002). Twelve concentrations were prepared with different levels of each drug (10 betalactam, 3 aminoglycosides, 3 macrolides, 4 sulfonamides, 3 tetracyclines and 3 quinolones). Table 1 summarizes the antimicrobial agents and the concentrations used for the preparation of the solutions. For each concentration, 16 replicates were prepared using antibiotic-free milk samples obtained from individual animals.

5. Statistical analysis: The results were achieved using SAS LOGISTIC procedure (SAS, 2001). The logistic regression for analyzing the effect of concentration of antimicrobial agents upon the relative absorption of "BT" and "BS" bioassays was the following:

$$L_{ij} = \beta_0 + \beta_1 A_i + \varepsilon_{ij}$$

Where: L_{ij} = lineal logistic model; β_0, β_1 = coefficients estimated for logistic regression model; A_i = antimicrobial concentration. ε_{ij} = residual error. The concordance coefficient (SAS, 2001) was applied as rank correlation between the observed and predicted relative absorption.

The detection capability of the photometric measurements of the "BT" and "BS" bioassays were calculated as the antimicrobial concentrations that produces 45 % of the maximum relative absorption (Luitz & Suhren, 1995; Luitz *et al.*, 1996).

RESULTS AND DISCUSSION

1. Detection capabilities: The results of applying logistic regression model to dose-response curves of the 26 antibiotics for "BT" and "BS" bioassays are shown in Table 2. Penicillins and cephalosporins had the highest values in the "β_1" coefficients, followed by tetracycline (for "BT" bioassay) and sulfonamides (for "BS" bioassay), indicating a high sensitivity of *G. stearothermophilus* for betalactam residues, since small increases in their concentrations produced changes in the system response. In contrast, residues of antibiotics belonging to other analyzed families (aminoglycosides, macrolides and quinolones) had lower values of the "β_1" coefficients. Therefore, ResScreen® system will not present an adequate detection level for these substances.

The adjustments made by the logistic model were acceptable, since the correlation coefficients ranged from 73.2 % for

erythromycin ("BS" bioassay) and 98.4 % for ciprofloxacin ("BS" bioassay) (Table 2).

The detection capabilities (CC_β) of the ResScreen® system, which were calculated using the equations of Table 2 and the criterion of percentage relative absorbance 45% proposed by Luitz *et al.* (1995), Suhren (1995) and Schliephake (1996), are shown in Table 3. The ResScreen® system had adequate CC_β for amoxycillin, ampicillin, cloxacillin, oxacillin, penicillin "G", cephalexin, ceftiofur and cefoperazone residues in milk (between MRL and 2 MRL). These levels were similar to those determined by Honkanen-

Table 1. Antimicrobial agent concentrations using for ResScreen® system detection capabilities.

Antibiotics	Bioassays	Concentrations (µg/l or *mg/l)
Beta-lactams		
Amoxycillin	BT - BS	0, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14
Ampicillin	BT - BS	0, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14
Cloxacillin	BT - BS	0, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70
Oxacillin	BT - BS	0, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 25
Penicillin "G"	BT - BS	0, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8
Cefadroxil	BT - BS	0, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220
Cephalexin	BT - BS	0, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220
Cefoperazone	BT - BS	0, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220
Ceftiofur	BT - BS	0, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220
Cefuroxime	BT - BS	0, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220
Tetracyclines		
Clortetracycline	BT	0, 0.5, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.6*
	BS	0, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6*
Oxytetracycline	BT	0, 25, 50, 75, 100, 125, 150, 175, 200, 300, 400, 500
	BS	0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 1, 1.2, 1.5*
Tetracycline	BT	0, 25, 50, 75, 100, 125, 150, 175, 200, 300, 400, 500
	BS	0, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.2, 1.5*
Sulfonamides		
Sulfadiazine	BT	0, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 80*
	BS	0, 80, 90, 100, 110, 120, 130, 140, 150, 160, 180, 200
Sulfamethazine	BT	0, 15, 17.5, 20, 25, 30, 35, 40, 45, 50, 60*
	BS	0, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8
Sulfamethoxazole	BT	0, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20*
	BS	0, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150
Sulfathiazole	BT	0, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20*
	BS	0, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120
Aminoglycosides		
Gentamycin	BT - BS	0, 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 800
Neomycin	BT - BS	0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2*
Streptomycin	BT - BS	0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5*
Macrolides		
Erythromycin	BT - BS	0, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 350
Lincomicin	BT - BS	0, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 300
Tylosin	BT - BS	0, 10, 20, 30, 40, 50, 60, 70, 80, 100, 120, 140
Quinolones		
Ciprofloxacin	BT - BS	0, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6*
Enrofloxacin	BT - BS	0, 1, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8, 3*
Marbofloxacin	BT - BS	0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5*

Buzalski & Reybroeck (1995) ($CC_{\beta Amoxicillin}$ = 6 g/l, $CC_{\beta Ampicillin}$ = 6μg/l, $CC_{\beta Cloxacillin}$ = 20μg/l, $CC_{\beta Penicillin}$ = 3μg/l and $CC_{\beta Ceftiofur}$ = 50μg/l); Luitz *et al.* (1996) ($CC_{\beta Ampicillin}$ = 3 μg/l, $CC_{\beta Cloxacillin}$ = 20 μg/l); Van Os & Beukers (1980) ($CC_{\beta Ampicillin}$ = 5-6 μg/l, $CC_{\beta Cloxacillin}$ = 25 -30μg/l, $CC_{\beta Penicillin}$ = 4 - 5μg/l) with the Delvotest® method. In addition, Zaadhof *et al.* (1997) ($CC_{\beta Cloxacillin}$ = 20 - 30 μg/l, $CC_{\beta Penicillin}$ = 2 - 3μg/l) and Althaus *et al.* (2001) ($CC_{\beta Amoxicillin}$ = 6 g/l, $CC_{\beta Ampicillin}$ = 6μg/l, $CC_{\beta Cloxacillin}$ = 51μg/l, $CC_{\beta Penicillin}$ = 2μg/l, $CC_{\beta Cephalexin}$ = 270μg/l and $CC_{\beta Ceftiofur}$ = 92 μg/l) calculated similar CC_{β} when the BRT® AiM method was used with ewe milk. For the Eclipse® method "100 ov", Montero *et al.* (2005) calculated similar CC ($CC_{\beta Amoxicillin}$ = 7 g/l, $CC_{\beta Cloxacillin}$ = 68μg/l, $CC_{\beta Oxacillin}$ = 28 μg/l, $CC_{\beta Penicillin}$ = 5 μg/l and $CC_{\beta Cephalexin}$ = 115μg/l) to those determined in this work.

Regarding sulfonamides, "BT" bioassay was not sensitive to detect residues of this group, while "BS" bioassay detected sulfadiazine (106 μg/l), sulfamethoxazole (78 μg/l) and sulfathiazole (115 μg/l) near their MRLs. In a previous study, Althaus *et al.*

Table 2. Summary of logistic regression model parameters of antibiotics in milk for ResScreen® system.

Antibiotics	BT bioassay			BS bioassay		
	β_0	β_1	C	β_0	β_1	C
Beta-lactams						
Amoxycillin	-6,8792	0,8992	92,6	-3,1714	0,8278	81,5
Ampicillin	-4,7931	0,6691	89,9	-4,4413	1,2072	88,3
Cloxacillin	-7,8820	0,2045	94,5	-6,3575	0,1674	92,6
Oxacillin	-6,4137	0,3765	90,4	-4,4731	0,3049	87,5
Penicillin "G"	-3,5494	1,0125	85,5	-5,2547	1,6494	92,3
Cefadroxil	-3,5502	0,0347	84,8	-5,5445	0,0340	89,3
Cephalexin	-5,1230	0,0600	94,0	-5,5463	0,0391	91,3
Cefoperazone	-3,5178	0,0518	89,6	-4,6683	0,0434	89,9
Ceftiofur	-2,7680	0,0449	81,6	-3,3739	0,0439	86,7
Cefuroxime	-4,2925	0,0435	92,6	-3,5709	0,0290	86,7
Tetracyclines						
Clortetracycline	-2,9333	0,0088	80,9	-3,4948	0,00123	87,6
Oxytetracycline	-4,0005	0,0262	89,3	-3,6682	0,00685	89,6
Tetracycline	-4,4139	0,0275	89,9	-4,4052	0,00816	92,1
Sulfonamides						
Sulfadiazine	-2,6766	0,00008	93,2	-2,9629	0,0339	88,8
Sulfamethazine	-1,6760	0,00007	89,1	-1,8302	0,00689	88,5
Sulfamethoxazole	-4,7884	0,00055	91,0	-0,9513	0,0202	86,3
Sulfathiazole	-5,7720	0,0006	89,1	-1,4983	0,0184	86,9
Aminoglycosides						
Gentamycin	-1,6709	0,0036	80,2	-3,2392	0,00547	87,8
Neomycin	-2,0165	0,0022	81,3	-4,6230	0,00486	93,8
Streptomycin	-4,2253	0,0019	81,9	-17,3530	0,00950	97,9
Macrolides						
Erythromycin	-3,5869	0,0122	89,8	-1,3677	0,00511	73,2
Lincomicin	-1,6333	0,0064	86,6	-1,0322	0,00473	83,0
Tylosin	-1,5683	0,0214	78,3	-1,0328	0,0199	74,5
Quinolones						
Ciprofloxacin	-3,5896	0,00147	95,0	-4,6805	0,0017	98,4
Enrofloxacin	-6,1819	0,0034	89,2	-2,0371	0,00157	74,6
Marbofloxacin	-4,4572	0,00131	89,3	-1,7160	0,00061	74,5

β_0, β_1 = *coefficients estimated for the logistic regression models; C: concordance coefficients.*

(2002) detected 260 µg/l of sulfadimethoxine and 110 µg/l of sulfamethoxazole using the Delvotest® "SP" method with ewe milk. Charm and Ruth (1993) observed a detection level of 1000 µg/l of sulfadiazine in cow milk. The Eclipse® "100ov" microbiological method detected 170 µg/l of sulfadimethoxine 750 µg/l of sulfamethazine and 250 µg/l of sulfathiazole (Montero *et al.*, 2005), values similar to the "BS" bioassay detection capabilities (Table 3).

In case of the tetracyclines, the "BT"bioassay showed detection capabilities in the range MRL-3MRL. Conversely, when analyzing milk samples containing these antibiotics by the "BS" bioassay, the detection capabilities calculated were higher (10 to 30 times MRL). In this regard, other microbiological methods yield higher detection levels for these antibiotics, such as BRT AiM® ($CC_{\beta Oxytetracycline}$ = 5500 µg/l, $CC_{\beta Tetracycline}$ = 6200 µg/l) Delvostest® "SP"

Table 3. ResScreen® system detection capabilities (µg/l) for antibiotics in milk.

Antibiotics	"BT" bioassay	"BS" bioassay	MRLs[a]
Beta-lactams			
Amoxycillin	8	5	4
Ampicillin	8	4	4
Cloxacillin	42	42	30
Oxacillin	19	17	30
Penicillin "G"	4	4	4
Cefadroxil	120	180	-
Cephalexin	96	150	100
Cefoperazone	80	120	50
Ceftiofur	75	90	100
Cefuroxime	113	140	-
Tetracyclines			
Clortetracycline	400	3300	100
Oxytetracycline	180	630	100
Tetracycline	180	620	100
Sulphonamides			
Sulfadiazine	41200	106	100
Sulfamethazine	3300	355	100
Sulfamethoxazole	9800	78	100
Sulfathiazole	10700	115	100
Aminoglycosides			
Gentamycin	640	700	100
Neomycin	1200	1100	1500
Streptomycin	2600	1900	200
Macrolides			
Erythromycin	350	390	40
Lincomicin	350	350	150
Tylosin	100	83	50
Quinolones			
Ciprofloxacin	2855	3120	100
Enrofloxacin	2000	1700	100
Marbofloxacin	3900	3800	75

[a] MRLs *(µg/l), EU Maximum Residues Limits.*

($CC_{\beta Oxytetracycline}$ = 320 µg/l, $CC_{\beta Tetracycline}$ = 590 µg/l) and Eclipse® "100ov" ($CC_{Chlortetracycline}$ = 1500 µg/l, $CC_{\beta Oxytetracycline}$ = 560 µg/l, $CC_{\beta Tetracycline}$ = 480 µg/l) according to Molina *et al.* (2003), Althaus *et al.* (2003) and Montero *et al.* (2005).

Finally, due to the low sensitivity of *G. stearothermohilus* to detect streptomycin, gentamicin, erythromycin and quinolones, these antibiotic residues could not be detected by ResScreen® system. These results were in accord with other commercial bioassays (BRT® AiM, Delvotest®, Eclipse®, etc.) using the same bacteria-test that did not detect these residues in milk samples (Heeschen & Blüthgen, 1995; Althaus *et al.*, 2002; Montero *et al.*, 2005; Linage *et al.*, 2007, Sierra *et al.*, 2009).

For those antimicrobial agents whose MRLs were published by the European Union, Figure 1 shows the detection pattern of the ResScreen® system. In this figure, a standardized MRL-scale was considered (Suhren *et al.*, 1996, Reicmuth *et al.*, 1997, Montero *et al.*, 2005) applying a logarithmic transformation to "detection capability/ MRL" for each antibiotic. The inner circle (1) corresponds to concentrations equivalent to 100 times "CC_{β} /MRL", circle 2 represents a level equivalent to 10 times "CC_{β} /MRL", the third circle (3) corresponds to the MRL and the outer circle (4) shows milk samples containing 0.1 times "CC_{β} /MRL". Thus, in a simple form is shown that ResScreen® system detects betalactams, sulfonamides,

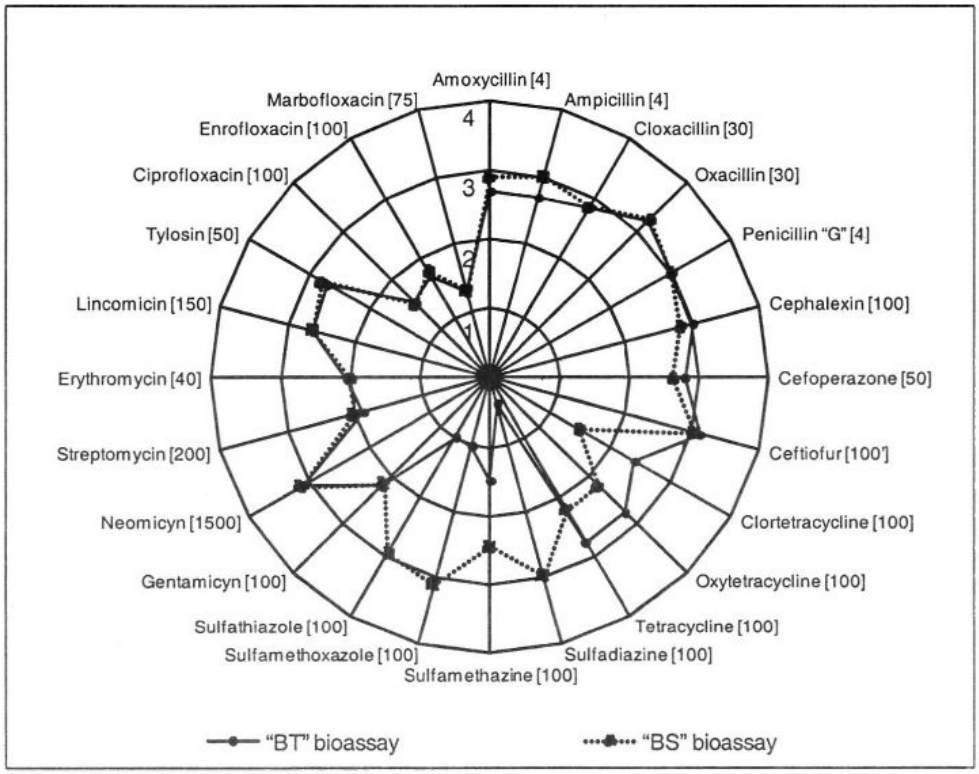

Fig. 1: Comparison of ResScreen system sensitivity for antibiotics in milk samples to EU-MRLs. Circle 1: 100 MRL, Circle 2: 10 MRL, Circle 3: MRL and Circle 4: 0.1 MRL. Maximum Residue Limit (MRL: µg/l) is mentioned in brackets.

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