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Novel microbiological system for antibiotic detection in ovine milk

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Abstract

This article presents a microbiological system composed of a "BT" bioassay (Beta-lactams and Tetracyclines) and a "QS" bioassay (Quinolones and Sulfonamides). The "BT" bioassay contains spores of *Geobacillus stearothermophilus*, bromocresol purple and cloramphenicol in a culture medium (incubation time: 2.45 h), while the "QS" bioassay uses spores of *Bacillus subtilis*, trifenyltetrazolium - toluidine blue and trimethoprim in a suitable culture medium (incubation time: 5.5 h). The detection capability (CC_β) of 27 antimicrobial agents in ovine milk were determined by logistic regression models. Thus, the "BT" bioassay detects amoxycillin, ampicillin, penicillin "G", cloxacillin, oxacillin, cephalexin, cefoperazone, ceftiofur, chlortetracycline, oxytetracycline, tetracycline, neomycin, gentamicin and tylosin, while "QS" bioassay detects: ciprofloxacin, enrofloxacin, marbofloxacin, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfathiazole, erythromycin, lincomycin and spiramycin at levels close to their respective Maximum Residue Limits. The simultaneous use of both bioassays detects a large number of antibiotics in milk given each method's adequate complementary sensitivity.

Keywords: ovine milk; system; bioassay; detection

1. Introduction

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In recent years, increased use of antibiotics to treat mastitis and other diseases of small ruminants was observed due to the intensification of milk production (Buswell and Barber, 1989).

The presence of antibiotic residuals in milk poses a potential risk for the consumers as they may cause allergic type reactions, and may interfere with intestinal flora and the development of resistance to antibiotics (Demoly and Romano, 2005; Dewdney et al., 1991; Currie et al., 1998; Wilke et al., 2005). Furthermore, antibiotic residues in milk can lead to important losses in fermented products, such as cheese-making (Berruga et al., 2007; Brady and Katz, 1988; Mourot and Loussourorn, 1981; Packham et al., 2001). Therefore, monitoring antibiotic residues is very important in controlling food safety. For these reasons, several control authorities such as the European Union (Council Directive, 2009) and Codex (Codex Alimentarius, 2009) determine the Maximum Residue Level (MRL) for the presence of specified veterinary residues in milk.

To this end, several commercially available tests have been developed for the swiftly and precisely detect of the presence of antibiotic residuals in milk (Toldra and Reig, 2006). Many of the screening tests are based on the inhibition of *G. stearothermophilus* subsp. *calidolactis* caused by the presence of drug residues. However, this bacteria does not have sensitivity to detect many of the antibiotics used to treat livestock such as quinolones (Montero et al., 2005), spiramycin, lincomycin (Linage et al., 2007), erythromycin and streptomycin (Molina et al., 2003; Althaus et al., 2002, 2003).

In addition, rapid methods are specific to small groups of antibiotics, but cannot increase the number of molecules to be controlled (Althaus et al., 2001; Roca et al., 2009).

Given the absence of a single ideal screening method that is sensitive to a large number of antimicrobial agents in ovine milk, the objective of this study was to evaluate the

application of a microbiological system that uses two bacteria test (*G. stearothermophilus* and *B. subtilis*) to detect a larger number of antibiotics in milk and to ensure consumer food safety.

2. Materials and methods

2. 1. Preparation of microplates

The "BT" bioassay (*G. stearothermophilus*): Plate Count Agar (Difco®, Ref. 247940) culture medium (6.25 g/l casein peptone, 2.25 g/l yeast extract and 15 g/l agar) fortified with glucose (10 g/l; Sigma®, Ref 158968) was used. The culture medium was sterilized to 121ºC for 15 min. Then, it was cooled to 50±1ºC and the pH was adjusted to a value of 7.0±0.1. Once prepared, the spores suspension of *G. stearothermophilus* subsp. *calidolactis* C-953 (10^7 spores/ml, Merck®, Ref. 1.11499), bromocresol purple indicator (0.05 mg/l, Mallinckrodt®, Ref. 2090) and chloramphenicol (400 μg/ml, Sigma Aldrich®, Ref. C0378) were added in accordance with Nagel et al. (2009).

The "QS" bioassay (*B. subtilis*): Müeller Hinton (38 g/l, Biokar Diagnostics®, Ref. BK048HA) culture medium fortified with glucose (10 g/l; Sigma Aldrich®, Ref. G7528), trimethoprim (400 mg/l; Sigma Aldrich®, Ref. T7883), 2,3,5-tripheyltetrazolium chloride (150 mg/l; Sigma Aldrich®, Ref. T8877) and toluidine blue (15 mg/l; Sigma Aldrich®, Ref. 198161) was employed. Once prepared, the culture medium was inoculated with the spore's suspension of *B. subtilis* BGA (Merck®, Ref. 1.10649) under sterile conditions in accordance with Nagel (2009).

Then 100 μl of the culture medium were added to each individual well of microtiter plate using an electronic pipette (Eppendorf Research® Pro). Next, these microplates were sealed with aluminized film and conserved at 4ºC until use.

2.2. Animals and ewe milk samples

The ewes were fed with natural pastures of *Melilotus albus*, *Trifolium repens* and *Lolium multiflorum*, during the lactation period. Individual samples were collected from 40 Pampinta (Milchschaff x Corriedale) ewes from the experimental farm at the Escuela de Agricultura Ganadería y Granja of the Universidad Nacional del Litoral in Argentina (south latitude: 31° 28', west longitude: 60° 55'). Animals did not receive any antimicrobial substances, and the samples were collected from ewes in the period between 30 and 90 days postpartum, from the recorder jar during morning milking and placed in 100 ml sterile plastic containers. Milk samples were kept at 4º C throughout the experiment.

2.3. Antimicrobial solutions and spiked samples

Drugs for the preparation of antimicrobial solutions were stored and handled according to the manufacturers' instructions before use. All the dilutions were prepared in 10 mL volumetric flasks at the time when analyses were carried out to avoid possible inconvenience due to instability. Antimicrobial solutions were prepared from the respective stock solution in a single step using antimicrobial-free milk (IDF, 2002), as determined by the "BT" and "QS" bioassays.

The dose-response curves of the antimicrobial agents were established in line with the Codex Alimentarius guidelines (Codex Alimentarius, 2010). To this end, 8 concentrations were prepared with different levels of each drug (Table 1). For each concentration, 24 replicates were prepared using antibiotic-free ovine milk samples obtained from individual animals. Then, 50 µl milk samples were added to the individual wells of the "BT" and "QS" Bioassays. Plates were sealed with adhesive bands and incubated at 64±1ºC for 2.5 h ("BT" Bioassay) and 40±1ºC for 5.5 h ("QS" Bioassay) according to the colour change of the negative samples. Visual interpretation was carried out by 3 qualified individuals and evaluated as "negative" (BT" bioassay:

yellow and "QS" bioassay: rose) or "positive" (BT" bioassay: purple and "QS" bioassay: blue). For the statistical calculations, those visual results that presented at least 2 similar interpretations were considered.

2.3. Detection capability (CC_β) and statistical analysis

To determine the detection capability (CC_β), 8 betalactams (amoxycillin, ampicillin, penicillin "G", cloxacillin, oxacillin, cephalexin, cefoperazone, ceftiofur), 3 aminoglycosides (gentamicina, neomycin, streptomycin), 4 macrolides (erythromycin, lincomicin, tylosin, spiramycin), 3 quinolones (ciprofloxacin, enrofloxacin, marbofloxacin), 6 sulfonamides (sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfathiazole) and 3 tetracyclines (chlortetracycline, oxytetracycline, tetracycline) were analyzed according to Codex Alimentarius guidelines (Codex Alimentarius, 2010).

The results were obtained using the SAS® Logistic procedure (SAS®, 2001). The logistic regression model was also used to calculate the detection limits, as follows:

$$L_{ij} = \text{logit}\,[P_{ij}] = \beta_0 + \beta_1\,[A]_i + \varepsilon_{ij} \qquad (1)$$

Where: L_{ij} = lineal logistic model; $[P_{ij}]$ = logit $[P_p/(1-P_p)]$: the probability of a "positive" response / probability of a "negative" response); β_0, β_1 = the coefficients estimated for the logistic regression models; $[A]_i$ = antimicrobial concentration. ε_{ij} = residual error. The concordance coefficient (SAS®, 2001) was applied as a rank correlation between the observed responses and the predicted probabilities.

The CC_β were estimated as concentrations at which 95% of the positive results (Codex Alimentarius, 2010).

3. Results and discussion

122 Table 2 shows the results obtained by applying the logistic regression model to the
123 visual interpretations of the "BT" and "QS" bioassays for the 27 antimicrobials
124 analyzed in sheep´s milk.
125 The "β_1" parameters indicate the slopes of the dose-response curves. Therefore, high
126 values of this coefficient show a good sensitivity of the bacteria test to detect a
127 particular antibiotic in milk.
128 The "BT" bioassay presents high "β_1" coefficients values to beta-lactam antibiotics,
129 tetracyclines, tylosin and neomycin, while the "QS" bioassay offers high values for this
130 coefficients for most beta-lactams (except cloxacillin, cefoperazone and ceftiofur®),
131 macrolides, quinolones and sulfonamides.
132 The high "β_1" coefficients values, which use *G. stearothermophilus* for the detection of
133 tylosin and beta-lactam antibiotics in ovine milk, were indicated with the BRT® AiM
134 (Molina et al., 2003), Delvotest® SP (Althaus et al., 2002), Charm Blue-Yellow (Linage
135 et al., 2007) and Eclipse® 100ov (Montero et al., 2005) methods. In addition, the last
136 two methods presented high "β_1" parameters to sulfonamides. For the "QS" bioassay,
137 Nagel (2009) indicated high "β_1" coefficients values when analyzing samples of cow's
138 milk fortified with sulfonamide.
139 The concordance coefficients obtained by applying of the logistic model were high for
140 both bioassays. They fell between 70.49% for amoxicillin ("BT" bioassay) and 91.67%
141 for sulfadimethoxine ("BT" bioassay), demonstrating the correct adjustment achieved
142 by the logistic model.
143 The detection capability (CC_β), calculated as concentrations which produce 95% of the
144 positive results in dose-response curves (Codex Alimentarius, 2010), are summarized in
145 Table 3.

As regards the beta-lactam antibiotics analyzed, the "BT" bioassay presented similar CC_{β} to the respective MRLs (except cefoperazone), while the "QS" bioassay detected only to penicillin residues at the MRL level. The detection capability for the "BT" bioassay for beta-lactams were similar to the values calculated for BRT® AiM ($CC_{\beta Amoxicillin}$ = 6 μg/l, $CC_{\beta Ampicillin}$ = 6 μg/l, $CC_{\beta Cloxacillin}$ = 51 μg/l, $CC_{\beta Penicillin}$ = 2 μg/l, $CC_{\beta Cephalexin}$ = 270 μg/l, $CC_{\beta Cefoperazone}$ = 92 μg/l and $CC_{\beta Ceftiofur}$ = 120 μg/l) for Molina et al. (2003), Eclipse® 100ov ($CC_{\beta Amoxicillin}$ = 7 μg/l, $CC_{\beta Cloxacillin}$ = 68 μg/l, $CC_{\beta Penicillin}$ = 5 μg/l, $CC_{\beta Cephalexin}$ = 115 μg/l and $CC_{\beta Cefoperazone}$ = 110 μg/l) for Montero et al. (2005) and Charm® Blue-Yellow ($CC_{\beta Ampicillin}$ = 5-6 μg/l, $CC_{\beta Cloxacillin}$ = 33-42 μg/l, $CC_{\beta Penicillin}$ = 3-4 μg/l, $CC_{\beta Cephalexin}$ = 160-202 μg/l, $CC_{\beta Cefoperazone}$ = 73-82 μg/l and $CC_{\beta Ceftiofur}$ = 96-107 μg/l) for Linage et al. (2007), which also used *G. stearothermophilus* as the bacteria test. However, Althaus et al. (2002) indicated lower detection capability when using the Delvotest® SP method with ovine milk samples ($CC_{\beta Amoxicillin}$ = 3 μg/l, $CC_{\beta Ampicillin}$ = 2 μg/l, $CC_{\beta Cloxacillin}$ = 18 μg/l, $CC_{\beta Penicillin}$ = 1 μg/l, $CC_{\beta Cephalexin}$ = 40 μg/l, $CC_{\beta Cefoperazone}$ = 20 μg/l and $CC_{\beta Ceftiofur}$ = 33 μg/l).

Of the three aminoglycosides analyzed, only neomycin residues were detected by the "BT" bioassay at levels close to the MRL (1500 μg/l), while gentamycin must be present at higher concentrations (450 μg/l) to be detected by this bioassay. Neither bioassay was able to detect streptomycin residues (5000 μg/l for "BT" bioassay and 4500 μg/l for "SQ" bioassay). It is necessary to emphasize that the BRT®AiM (630 μg/l of neomycin, 3700 μg/l of gentamycin and 6000 μg/l of streptomycin), Delvotest® SP (2600 μg/l of neomycin, 1200 μg/l of gentamycin and 6100 μg/l of streptomycin), Eclipse® 100ov (9100 μg/l of neomycin, 3140 μg/l of gentamycin and 10100 μg/l of streptomycin) and Charm® Blue-Yellow (444-542 μg/l of neomycin, 355-382 μg/l of

gentamycin and 3063-3593 μg/l of streptomycin) methods obtained appropriate detection capability for neomycin (except Eclipse® 100ov), high ones for gentamicin, but proved inadequate for streptomycin in ovine milk according to Althaus et al. (2002), Linage et al. (2007), Molina et al. (2003) and Montero et al. (2005), respectively.

For macrolides, Table 3 shows that the CC_β for the "QS" bioassay for erythromycin (60 μg/l), lincomycin (280 μg/l), tylosin (140 μg/l) and spiramycin (380 μg/l) were slightly above their respective MRLs, indicating good sensitivity for *B. subtilis* for that family of antibiotics in milk. On the contrary, "BT" bioassay presents a detection capability for tylosin (100 μg/l) closer to their MRL (50 μg/l) if compared to the "QS" bioassay. The low sensitivity of *G. stearothermophilus* to detect erythromycin (630 μg/l for BRT® AiM, 830 μg/l for Delvotest® SP, 750 μg/l for Eclipse® 100ov, and 444-522 μg/l for Charm® Blue-Yellow) and spiramycin (18100 μg/l for Eclipse® 100ov, and 1106-1346 μg/l for Charm Blue-Yellow) was pointed out by those authors.

Of the three quinolones tested, ciprofloxacin (160 μg/l) and enrofloxacin (230 μg/l) were detected by the "QS" bioassay at levels near their MRL (100 μg/l), while marbofloxacin residues must be present in milk at a higher level (280 μg/l) than the MRL (75 μg/l) to be detected by this method. In contrast, the "BT" bioassay was not sensitive to these antibiotics because it presented high CC_β for ciprofloxacin (2280 μg/l), enrofloxacin (2770 μg/l) and marbofloxacin (5540 μg/l) in ovine milk. It is noteworthy that Montero, Althaus et al. (2005) reported high CC_β for ciprofloxacin (5100 μg/l) and enrofloxacin (4000 μg/l) when using the Eclipse® 100ov method to analyze ovine milk samples fortified with quinolones. Similarly, Linage et al. (2007) reported a wide range (41000-46000 μg/l) for the enrofloxacin residues analyzed by the Charm® Blue-Yellow method.

194 Once again, these studies indicate that the use of these commercial methods containing
195 *G. stearothermophilus* is inadequate to control quinolones residues in ovine milk, and
196 that the use of another bacteria test (e.g., *B. subtilis*) is necessary.
197 Regarding sulfonamides, Table 3 shows that the "QS" bioassay presented similar
198 detection capability ($CC_{\beta Sulfadiazine}$ = 157 μg/l, $CC_{\beta Sulfadimethoxine}$ = 136 μg/l, $CC_{\beta Sulfamerazine}$ =
199 115 μg/l, $CC_{\beta Sulfamethazine}$ = 200 μg/l, $CC_{\beta Sulfamethoxazole}$ = 123 μg/l and $CC_{\beta Sulfathiazole}$ = 122
200 μg/l) to the MRLs. However, the "BT" Bioassay did not provide good limits for this
201 family of antibiotics because there was no trimethoprim in the culture medium (Nagel et
202 al., 2009).
203 These limits were similar to those reported for the Charm® Blue-Yellow test
204 ($CC_{\beta Sulfadimethoxine}$ = 101-119 μg/l; $CC_{\beta Sulfamethazine}$ = 309-328 μg/l, $CC_{\beta Sulfathiazole}$ = 122-151
205 μg/l) by Linage et al. (2007), but were lower than the levels obtained for Eclipse® 100ov
206 ($CC_{\beta Sulfadimethoxine}$ = 170 μg/l; $CC_{\beta Sulfamethazine}$ = 750 μg/l, and $CC_{\beta Sulfathiazole}$ = 250 μg/l)
207 reported by Montero et al. (2005) when using *G. stearothemophilus* instead of *B.*
208 *subtilis*. Nevertheless, Althaus et al. (2002) calculated lower detection capability
209 ($CC_{\beta Sulfadiazine}$ = 88 μg/l and $CC_{\beta Sulfamethoxazole}$ = 44 μg/l) than those obtained in this work
210 (Table 3) when analyzing ovine milk samples by the Delvotest® SP method.
211 To synthesize, the Figure 1 shows the detection pattern by the simultaneous
212 implementation of "BT" and "QS" bioassays. This scale was constructed by applying
213 the logarithmic transformation to CC_{β}/MRL for each antimicrobial. The interior, central
214 and outer polygons corresponds to concentrations equivalent to 10 MRL, MRL, and 0.1
215 MRL, respectively.
216 This figure summarizes the adequate detection capability of the microbiological system,
217 since most of the antibiotics have detection capability near their corresponding MRLs,

with the exception of streptomycin. It is noted that the CC_{β} of the different antibiotics analyzed by this microbiological system are located close to central polygon (MRL).

4. Conclusions

The microbiological system consists of two bioassays using *G. stearothermophilus* and *B. Subtilis*, which can detect a large number of antibiotics in milk (beta-lactams, quinolones, sulfonamides, tetracyclines, erythromycin, lincomycin, neomycin, spiramycin and tylosin) if compared with other currently used microbiological methods. This improved detection of antibiotic residues is achieved by using two bacteria tests with complementary sensitivity to detect different antibiotics.

Therefore, this microbiological system proves to be a valuable tool to control the quality of ovine milk. The implementation of this system with two bacteria tests enables a more rigorous control of antibiotic residues in milk and, consequently, helps protect consumers' health.

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Table 1

Antimicrobial agent concentrations using for microbiological system.

Antibiotics	"BT" bioassay	"QS" bioassay
Betalactams		
Amoxycillin	0, 1, 2, 3, 4, 5, 6, 8	0, 1, 2, 4, 6, 8, 10, 12
Ampicillin	0, 1, 2, 3, 4, 5, 6, 8	0, 5, 10, 20, 30, 40, 50, 60
Cloxacillin	0, 10, 15, 20, 25, 30, 40, 60	0, 50, 100, 150, 200, 250,300, 400
Oxacillin	0, 2, 5, 10, 15, 20, 25, 30	0, 25, 50, 100, 150, 200, 250, 300
Penicillin "G"	0, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0	0, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0
Cephalexin	0, 25, 50, 75, 100, 150, 200, 300	0, 25, 50, 100, 125, 150, 200, 300
Cefoperazone	0, 50, 75, 100, 150, 200, 300, 400	0, 50, 100, 125, 150, 200, 300, 400
Ceftiofur®	0, 25, 50, 75, 100, 150, 200, 300	0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8*
Aminoglycosides		
Gentamycin	0, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8*	0, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 1.0*
Neomycin	0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0*	0, 2, 3, 4, 5, 6, 7, 8*
Streptomycin	0, 1, 2, 3, 4, 5, 6, 7*	0, 1, 2, 3, 4, 5, 6, 7*
Macrolides		
Erythromycin	0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.4*	0, 10, 20, 30, 40, 50, 60, 70
Lincomicin	0, 0.1, 0.15, 0.20, 0.25, 0.30, 0.40, 0.50*	0, 0.10, 0.15, 0.20, 0.25, 0.30, 0.40, 0.50*
Tylosin	0, 25, 50, 75, 100, 125, 150, 200	0, 60, 80, 100 ,120, 140, 160, 180
Spiramycin	0, 1, 2, 3, 4, 5, 6, 7*	0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7*
Quinolones		
Ciprofloxacin	0, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0*	0, 50, 100, 150, 200, 250, 300, 400
Enrofloxacin	0, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0*	0, 50, 100, 150, 200, 250, 300, 400
Marbofloxacin	0, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 7.0*	0, 50, 100, 150, 200, 250, 300, 400
Sulphonamides		
Sulfadiazine	0, 10, 20, 30, 40, 50, 60, 80*	0, 50, 100, 200, 300, 400, 500, 600
Sulfadimethoxine	0, 0.5, 0.8, 1.0, 1.3, 1.5, 2.0, 3.0*	0, 50, 100, 200, 300, 400, 500, 600
Sulfamerazine	0, 10, 15, 20, 25, 30, 35, 40*	0, 50, 100, 200, 300, 400, 500, 600
Sulfamethazine	0, 10, 20, 30, 40, 50, 60, 80*	0, 50, 100, 200, 300, 400, 500, 600
Sulfamethoxazole	0, 5, 10, 15, 20, 25, 30, 35*	0, 50, 100, 200, 300, 400, 500, 600
Sulfathiazole	0, 5, 10, 15, 20, 25, 30, 35*	0, 50, 100, 200, 300, 400, 500, 600
Tetracyclines		
Clortetracycline	0, 50, 100, 150, 200, 300, 400, 500	0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7*
Oxytetracycline	0, 25, 50, 75, 100, 150, 200, 300	0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7*
Tetracycline	0, 25, 50, 75, 100, 150, 200, 300	0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2*

Units: μg/l or *mg/l.

Table 2

Summary of logistic regression model parameters of antibiotics in ovine milk for microbiological system.

Antibiotics	"BT" Bioassay			"QS" Bioassay		
	β_0	β_1	C	β_0	β_1	C
Betalactams						
Amoxycillin	-4,950	2,123	70,49	-4,508	1,324	73,79
Ampicillin	-5,652	2,424	74,63	-6,055	0,723	88,29
Cloxacillin	-4,771	0,308	75,47	-5,406	0,036	66,95
Oxacillin	-3,064	0,402	73,02	-5,870	0,133	86,30
Penicillin "G"	-10,975	5,270	77,43	-10,320	3,707	71,34
Cephalexin	-3,237	0,048	79,14	-8,196	0,079	75,82
Cefoperazone	-11,619	0,084	75,72	-9,332	0,046	75,71
Ceftiofur®	-11,421	0,125	88,82	-5,722	0,026	75,54
Aminoglycosides						
Gentamycin	-7,959	0,024	74,06	-14,330	0,026	77,02
Neomycin	-6,143	0,007	79,30	-16,381	0,003	78,27
Streptomycin	-8,749	0,002	86,32	-11,179	0,003	81,25
Macrolides						
Erythromycin	-9,732	0,056	78,86	-13,493	0,289	78,23
Lincomicin	-11,560	0,044	74,27	-12,445	0,055	78,87
Tylosin	-7,572	0,104	76,69	-132,074	0,951	89,08
Spiramycin	-8,380	0,003	77,42	-10,915	0,036	86,38
Quinolones						
Ciprofloxacin	-8,679	0,005	87,03	-22,162	0,152	88,26
Enrofloxacin	-9,809	0,005	86,33	-13,963	0,071	86,56
Marbofloxacin	-11,628	0,003	87,17	-11,672	0,051	75,76
Sulphonamides						
Sulfadiazine	-4,956	0,000	84,56	-5,850	0,056	80,64
Sulfadimethoxine	-16,157	0,001	91,67	-4,449	0,054	78,41
Sulfamerazine	-19,487	0,001	86,32	-4,494	0,065	76,74
Sulfamethazine	-20,267	0,001	92,65	-3,769	0,034	73,58
Sulfamethoxazole	-18,659	0,001	90,78	-5,183	0,066	79,15
Sulfathiazole	-20,429	0,001	89,46	-3,749	0,055	79,78
Tetracyclines						
Clortetracycline	-8,730	0,043	85,6	-9.254	0.026	82.4
Oxytetracycline	-6,611	0,074	72,65	-9,827	0,022	72,38
Tetracycline	-6,081	0,058	70,55	-8,053	0,013	76,67

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

Table 3

Microbiological system detection capability (μg/l) for antibiotics in milk.

Antibiotics	"BT" Bioassay [a]	"QS" Bioassay [a]	MRL[b]
Betalactams			
Amoxycillin	4	6	4
Ampicillin	4	12	4
Cloxacillin	25	232	30
Oxacillin	15	66	30
Penicillin "G"	3	4	4
Cephalexin	128	141	100
Cefoperazone	174	266	50
Ceftiofur®	115	328	100
Aminoglycosides			
Gentamycin	450	670	100
Neomycin	1360	6700	1500
Streptomycin	5000	4500	200
Macrolides			
Erythromycin	230	60	40
Lincomicin	330	280	150
Tylosin	100	140	50
Spiramycin	4280	380	200
Quinolones			
Ciprofloxacin	2280	160	100
Enrofloxacin	2770	230	100
Marbofloxacin	5540	280	75
Sulphonamides			
Sulfadiazine	53000	157	100
Sulfadimethoxine	1300	136	100
Sulfamerazine	23000	115	100
Sulfamethazine	35000	200	100
Sulfamethoxazole	17000	123	100
Sulfathiazole	17000	122	100
Tetracyclines			
Clortetracycline	271	470	100
Oxytetracycline	129	570	100
Tetracycline	154	840	100

[a] Detection capabilities estimated as concentrations at which 95% of the positive results

[b] MRLs (μg/l).

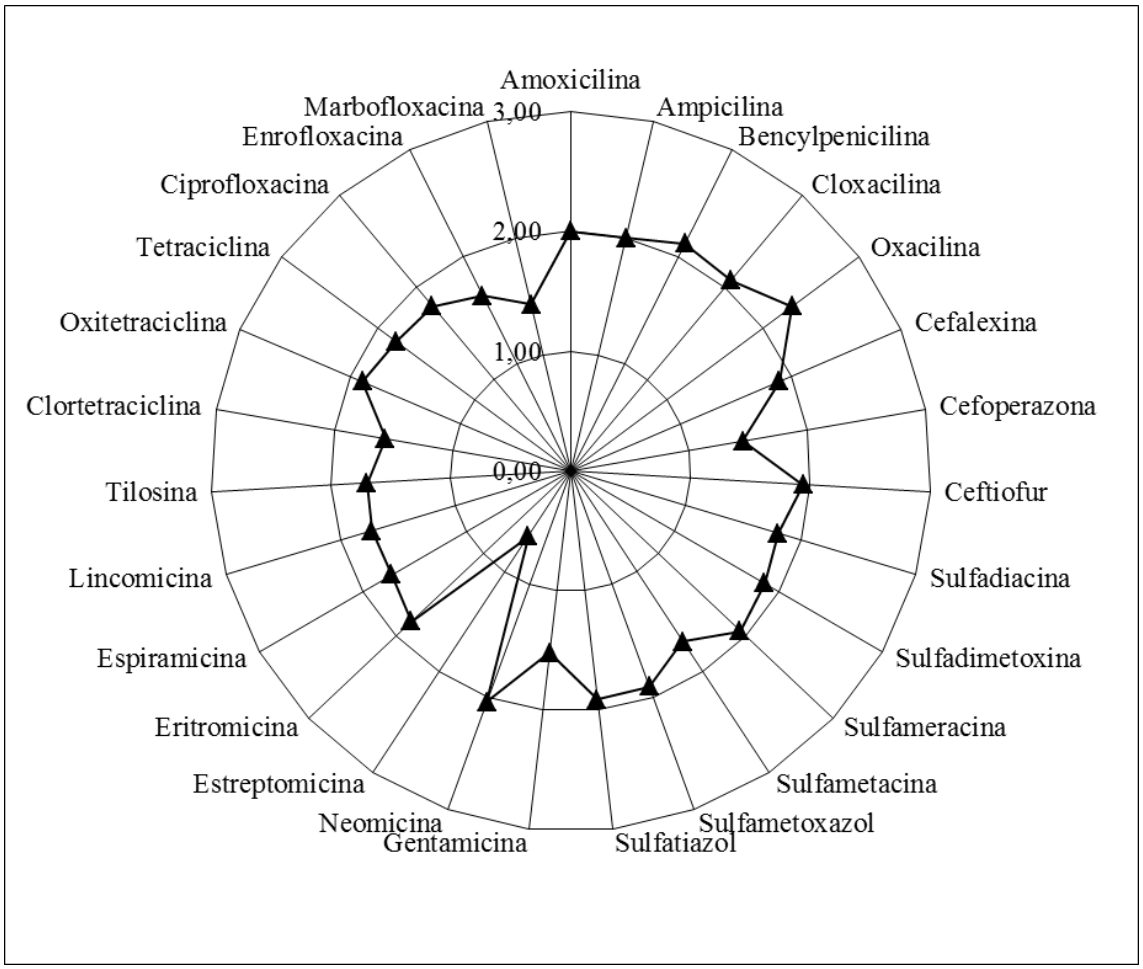

Fig. 1. Detection pattern by simultaneous implementation of BT and QS bioassays. Line 1: 10 CC_{β}/LMR, Line 2: CC_{β}/LMR and Line 3: 0.1 CC_{β}/LMR.
Note: The figure uses the lowest CC_{β} of antibiotics listed in Table 3.