Detection limits of four antimicrobial residue screening tests for β -lactams in goat's milk

D. Sierra,* A. Sánchez,†¹ A. Contreras,† C. Luengo,* J. C. Corrales,† C. T. Morales,* C. de la Fe,† I. Guirao,* and C. Gonzalo‡

*Laboratorio Agroalimentario y de Sanidad Animal, Consejería de Agricultura y Agua, 30120 Murcia, Spain

†Dpto. de Sanidad Animal, Facultad de Veterinaria, Universidad de Murcia, 30120 Murcia, Spain

‡Dpto. de Producción Animal, Facultad de Veterinaria, Universidad de León, 24071 León, Spain

ABSTRACT

This study was conducted to compare the detection limits (DL) of several antibiotic residue screening tests with the maximum residue limits (MRL) authorized by the EU according to the guidance for the standardized evaluation of microbial inhibitor tests of the International Dairy Federation. Composite antibiotic-free milk samples from 30 primiparous Murciano-Granadina goats in good health condition were used to prepare test samples spiked with different concentrations of each antimicrobial. In total, 5,760 analytical determinations of 10 β -lactam antibiotics (penicillin-G, ampicillin, amoxicillin, cloxacillin, oxacillin, dicloxacillin, cefadroxyl, cefalexin, cefoperazone, and cefuroxime) were performed using 4 antibiotic residue screening tests: the brilliant black reduction test BRT AiM (AiM-Analytik in Milch Produktions-und Vertriebs GmbH, München, Germany), Delvotest MCS (DSM Food Specialties, Delft, the Netherlands), Eclipse 100 (ZEU-Immunotec SL, Zaragoza, Spain), and the Copan Milk Test (CMT; Copan Italia SpA, Brescia, Italy). For each method, we estimated the detection limits of the antimicrobial agents using a logistic regression model. Using the CMT and Delvotest on samples spiked with the 8 antibiotics for which MRL were available, DL were at or below the MRL. The BRT test provided DL at or below the MRL for all of the agents except cefalexin, whereas the Eclipse 100 method failed to detect 4 antibiotics (ampicillin, amoxicillin, cloxacillin, and cefoperazone) at MRL or below. Logistic regression-determined levels of agreement were highest for the CMT method (98.6 to 100%) and lowest for Eclipse 100 (66.3 to 100%). In general, agreement levels indicated good correlation between observed results and those predicted by logistic regression. The lowest b values (closely related to test sensitivity) were recorded for the cephalosporins

(0.074 to 0.430) and highest for penicillin G, ampicillin, and amoxicillin (11.270 to 11.504). Delvotest and CMT best fulfilled IDF criteria for the ideal test for detecting antibiotic residues in milk.

Key words: β -lactam, goat's milk, antibiotic residue, detection limit

INTRODUCTION

The possible presence of antimicrobial residues (AR) in milk poses a risk for consumers because these may cause allergic reactions, intestinal dysbiosis, or even the emergence of resistant bacterial populations (Allison, 1985; Dewdney et al., 1991). Besides consequences for consumers, the presence of AR in milk can cause problems for producers because residues may impair bacterial fermentation processes (Mourot and Loussouarn, 1981) when milk is used to produce fermented dairy products. This problem especially affects sheep and goat's milk because they are often used to produce cheese. Given possible repercussions on public health and the dairy industry, official limits exist for AR and European Union (EU) legislation (European Union, 1990) stipulates maximum residue limits (MRL) for defined antimicrobials. Producers and the dairy industry may also suffer economic losses if a false positive AR result is obtained in an uncontaminated milk sample, and in some countries this may even have legal consequences. It is therefore important that besides showing high specificity, an antimicrobial residue screening test (ARST) should be sufficiently sensitive to detect minimal amounts of antimicrobials but not so sensitive that false-positive results are easily produced.

Although β -lactam antimicrobials are widely used in dairy goats and other dairy ruminants and milk testing programs in dairy goat herds include the determination of several residues in bulk tank milk samples, there is little information in the literature on ARST in goats. Zeng et al. (1996) compared the Delvotest P and Penzyme tests with a standard reference test (BsDA) and concluded that these tests were sensitive and reli-

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¹Corresponding author: asanlope@um.es

able for detecting AR in individual goat milk samples. These same authors (Zeng et al., 1998) also assessed the use of the SNAP test, Charm BsDA, Charm II, and LacTeck for ARST in goat's milk and found that the first 3 were sensitive and reliable for detecting AR but their high sensitivity could lead to false-positive results when AR were below the permitted, or tolerance, level. In contrast, the LacTeck B-L failed to detect tolerance levels of several antimicrobials, including penicillin G, in goat's milk. Given the devastating consequences of false positives (Cullor, 1992), in a previous study we assessed 9 different ARST commonly used in the United States for cow's milk on individual goat's milk samples (Contreras et al., 1997). These tests covered a variety of analytical principles for assaying β -lactam and ceftiofur residues and we were able to conclude that they were capable of adequately identifying goat's milk that was free of antimicrobial residues.

Most screening methods for AR detection in milk are based on inhibiting the growth of *Geobacillus stearothermophilus* var. *calidolactis* and have been developed and validated for use in cow's milk. Although some authors have assessed the use of screening kits in sheep's milk (Althaus et al., 2003a,b; Berruga et al., 2003; Molina et al., 2003; Montero et al., 2005; Linage et al., 2007), most of the information available is based on results obtained in cow's milk. The present study was designed to compare 4 commercial screening tests and determine their detection limits (DL) for 10 β -lactams in goat's milk.

MATERIALS AND METHODS

Animals and Milk Samples

Composite milk samples from 30 Murciano-Granadina goats in good health reared on an organic farm were used for the study. To ensure that the milk collected was antibiotic-free, we adapted the requirements of the guidance on the standardized evaluation of microbial inhibitor tests (IDF, 1999). Thus, the goats selected were primiparous, in midlactation and had never been given any form of antimicrobial treatment or feed supplements. Composite milk samples (200 mL) from the 30 selected goats were obtained on the morning of milking and transported refrigerated to the laboratory. The samples were then mixed to obtain AR-free milk, divided into 100-mL aliquots and frozen (-20°C) until used to prepare the samples spiked with the different antimicrobial agents. The animals' health status was good, including no clinical or subclinical IMI (including *Mycoplasma* spp.). To ensure mammary health (especially in terms of checking for the possible presence of β -lactamase producing organisms), 1 and 2 d

before selecting the animals, individual milk samples were obtained for bacteriological analysis according to the recommendations of the National Mastitis Council (Harmon et al., 1990). This involved collecting 10 mL of foremilk aseptically from each udder half. Teats were scrubbed with cotton saturated with 70% ethanol and the first 3 streams of milk were discarded. Ten microliters of each sample were plated on blood agar plates (5% washed sheep erythrocytes). The plates were incubated aerobically at 37°C and examined at 24 h and 48 h. The presence of 500 cfu/mL was taken to indicate an IMI. Similarly, milk samples were tested for *Mycoplasma* spp. 2 weeks before animal selection by inoculating the samples in modified PH medium broth followed by incubation in a high-humidity CO_2 (5%) atmosphere for 2 d, filtering and subpassaging in broth, and incubating for an additional 9 d. Thus, all the goats finally selected had tested negative for IMI, including *Mycoplasma* spp., and their milk SCC was lower than 256×10^3 .

Antimicrobial Spiked Samples and Residue Screening Tests

Milk samples containing the following β -lactam antimicrobials were prepared following the recommendations of IDF (1999): penicillin-G, ampicillin, amoxicillin, cloxacillin, oxacillin, dicloxacillin, cefadroxyl, cefalexin, cefoperazone, and cefuroxime. These drugs were stored and handled according to the manufacturer's instructions before use. Drugs were dissolved (1 mg/mL) in distilled water and 8 different dilutions of spiked milk samples prepared for each antimicrobial, including a blank (Table 1). All dilutions were prepared in 100-mL volumetric flasks on the day of analysis, to avoid possible errors caused by the instability of the solutions. For each antimicrobial, we tested 18 replicates of each dilution using the 4 ARST, to give a total of 5760 analytical determinations.

The screening tests assessed were: the brilliant black reduction test BRT AiM (**BRT**; AiM-Analytik in Milch Produktions-und Vertriebs GmbH, München, Germany), the Delvotest MCS (Delvotest; DSM Food Specialties, Delft, the Netherlands), the Eclipse 100 (Eclipse; ZEU-Inmunotec SL, Zaragoza, Spain) and the Copan Milk Test (**CMT**; Copan Italia SpA, Brescia, Italy). These tests are all based on detecting inhibition of the growth of spores of *Geobacillus stearothermophilus* var. *calidolactis* C (**Gsc**). The test medium is a mixture of nutrients, test bacteria (*Gsc*), a colored indicator (brilliant black for BRT and bromocresol purple for the other ARST) and other supplements. When the milk sample contains no residues or these are under the detection limits, the spores germinate,

Table 1. List of the β -lactams used in this study (all supplied by Sigma, St. Louis, MO), their commercial reference numbers, and final concentrations in the spiked milk samples

Antimicrobial	Reference no.	Concentrations ($\mu\text{g/kg}$)
Penicillin G	P-3032	0, 0.5, 1, 2, 3, 4, 5, 6
Ampicillin	A-9518	0, 0.5, 1, 2, 3, 4, 5, 6
Amoxicillin	A-8523	0, 0.5, 1, 2, 3, 4, 5, 6
Cloxacillin	C-9393	0, 5, 7.5, 10, 20, 30, 40, 50
Dicloxacillin	D-9016	0, 5, 7.5, 10, 20, 30, 40, 50
Oxacillin	O-1002	0, 5, 7.5, 10, 20, 30, 40, 50
Cefalexin	C-4895	0, 20, 40, 60, 80, 100, 200, 400
Cefadroxyl	C-7020	0, 20, 40, 60, 80, 100, 200, 400
Cefuroxime	C-4417	0, 10, 20, 50, 80, 100, 200, 400
Cefoperazone	C-4292	0, 10, 20, 50, 80, 100, 200, 400

grow and their metabolic activity makes the indicator change color. In the presence of antimicrobial residues, no bacterial activity will be detected and the indicator color will remain unchanged (blue or purple). The color change can be visually or photometrically assessed. All ARST were carried out in 96-well microtiter plates according to the manufacturer's instructions. Briefly, the spiked milk samples (100 μL each) were inoculated in the wells containing culture medium for *Gsc* and a pH indicator, including a negative and a positive control. The plates were then carefully sealed and placed in a water bath and incubated at $64 \pm 1^\circ\text{C}$ for 3 h. Results were interpreted visually for CMT and photometrically for the other 3 tests. A spectrophotometer was used at measuring and reference wavelengths of 450 and 620 nm for the BRT and 590 and 650 nm for Eclipse. The DelvoScan Reader was used for Delvotest. Copan Milk Test results were separately interpreted visually by 2 technicians and to ensure the validity of the results from the 2 technicians, the agreement between their results was estimated using the kappa value (Thrusfield, 1995).

Statistical Analysis

The DL of each test was determined for each antimicrobial agent by logistic regression using the Logistic procedure of SAS (SAS Institute, 1998). The response observed was considered binary and classified as "positive," "dubious," or "negative." The logistic regression model used was

$$L_{ij} = \text{logit } [P_{ij}] = a_0 + bAC_i + \varepsilon_{ij},$$

where logit = linear logistic model, that is, $\ln[P_{ij}/(1 - P_{ij})]$; P_{ij} = probability of "negative" versus "positive plus dubious" results; AC = antimicrobial concentration; a = intercept; b = slope; and ε_{ij} = residual error. Agreement coefficients were estimated as rank correlation between observed and predicted results (Althaus et

al., 2003b). The DL was estimated as the concentration at which 95% of results were positive (IDF, 1999).

RESULTS AND DISCUSSION

Tables 2, 3, 4, and 5 summarize the results of the logistic regression model in terms of relative frequencies of each ARST result and the DL value recorded. For 8 out of the 10 antibiotics for which EU MRL were available, CMT and Delvotest showed DL at or below the MRL. The BRT results indicated detection limits at or below the MRL for these 8 antibiotics except cefalexin, whereas Eclipse failed to detect 4 antibiotics (ampicillin, amoxicillin, cloxacillin and cefoperazone) at levels corresponding to MRL or below. Logistic regression agreement levels were highest for CMT (98.6 to 100%) and lowest for Eclipse (66.3 to 100%) indicating overall good correlation between observed results and those predicted by logistic regression. The coefficient b (slope) of the logistic regression line is closely related to the sensitivity of a test to a given antimicrobial agent. Using all 4 ARST, lowest b values were obtained for the cephalosporins (0.074 to 0.430) and highest values for penicillin G, ampicillin, and amoxicillin (11.270 to 11.504). Figures 1, 2, 3, and 4 show the DL results obtained for the 4 ARST assessed in relation to MRL represented as the detection pattern suggested by the IDF (1999). Thus, the ideal test should be capable of detecting $1 \times$ MRL of all antimicrobials of concern, which is indicated in the figures by the middle circle. The DL of the test under study is indicated by the circle in bold. It may be observed that Delvotest and CMT best fulfilled IDF criteria for the ideal AR test.

Delvotest is a simple, rapid, and economic ARST, and because of these features it is commonly used to detect antimicrobial residues in cow's milk. Despite scarce data on the use of this test to detect antibiotic residues in goat's milk, it is presently widely used for this purpose (Zeng et al., 1996). In separate assessments of Delvotest-P by Zeng et al. (1996) and Contreras et

Table 2. Logistic regression model variables and detection limits of the β -lactams obtained using Delvotest MSC (DSM Food Specialties, Delft, the Netherlands) in goat's milk considering a binary response variable

Antimicrobial	Intercept (a)	Slope (b)	Concordance coefficients (%)	Detection limits ($\mu\text{g/kg}$)	MRL ¹ ($\mu\text{g/kg}$)
Penicillin G	-16.946	11.270	100	2	4
Ampicillin	-40.175	11.496	80	4	4
Amoxicillin	-40.175	11.496	80	4	4
Cloxacillin	-23.373	1.134	98.6	23	30
Dicloxacin	-23.979	1.262	98.9	21	30
Oxacillin	-20.164	3.216	100	7	30
Cefalexin	-21.000	0.420	100	57	100
Cefadroxyl	-21.000	0.420	100	57	—
Cefuroxime	-11.110	0.316	100	44	—
Cefoperazone	-11.110	0.316	100	44	50

¹MRL = maximum residue limits.

al. (1997) using individual goat's milk samples, no relationship was detected between the SCC and the results of the antibiotic detection test, despite contradictory results obtained in terms of false-positive results. Thus, Zeng et al. (1996) obtained 7% false-positive results whereas we observed no false positives (Contreras et al., 1997). Using Delvotest in sheep's milk, Althaus et al. (2003a) recorded 2.3% false-positive results and observed lower proportions of total solids and higher SCC in these false positive samples. Recently, Stead et al. (2008) validated the Delvotest SP-NT in cow's milk and no false positives or negatives were obtained. These authors also determined that this test could be used to AR screen a wide range of milk types in terms of fat content or SCC including goat's milk, with the exception of milk of low pH (spoiled milk).

The detection limits of Delvotest in cow's milk have been visually assessed by several authors. Le Breton et al. (2007) compared visual and scanner readings, concluding that the automated reader (scanner) is a useful and convenient tool. Our results indicate that Delvotest MSC is useful for detecting β -lactam residues in goat's milk because it was able to detect residues of

all the β -lactams included in our study below or at the MRL (Table 2 and Figure 1). The DL for ampicillin and amoxicillin were in line with their EU MRL whereas those determined for the remaining agents were below the MRL. These results are in agreement with those reported by Althaus et al. (2003b) who estimated the Delvotest SP detection limits in sheep's milk of the photometric measurement of 4 β -lactams (penicillin G, amoxicillin, ampicillin, and cloxacillin). Although in both this study and our study residues of these 4 antibiotics below EU MRL were detected, our results were closest to the MRL (2 $\mu\text{g/kg}$ in our study versus 1 $\mu\text{g/kg}$ for penicillin G; 4 $\mu\text{g/kg}$ versus 2 $\mu\text{g/kg}$ for ampicillin; 4 $\mu\text{g/kg}$ versus 3 $\mu\text{g/kg}$ for amoxicillin; and 23 $\mu\text{g/kg}$ versus 18 $\mu\text{g/kg}$ for cloxacillin).

The BRT is another test based on microbiological inhibition widely used for the detection of AR in milk and has been marketed by different manufacturers under several trade names. Its DL for antibiotic residues has been widely established in cow's milk and there are a few reports available for sheep's milk, whereas no information exists for goat's milk. Using the BRT in sheep's milk, Althaus et al. (2003a) observed 3.7%

Table 3. Logistic regression model variables and detection limits of the β -lactams obtained using the Brilliant Reduction Test (BRT AiM, AiM-Analytik in Milch Produktions-und Vertriebs GmbH, München, Germany) in goat's milk considering a binary response variable

Antimicrobial	Intercept (a)	Slope (b)	Concordance coefficients (%)	Detection limits ($\mu\text{g/kg}$)	MRL ¹ ($\mu\text{g/kg}$)
Penicillin G	-16.946	11.270	100	2	4
Ampicillin	-28.753	11.504	100	3	4
Amoxicillin	-40.175	11.496	80	4	4
Cloxacillin	-30.280	1.215	100	27	30
Dicloxacin	-26.783	3.705	100	8	30
Oxacillin	-26.783	3.705	100	8	30
Cefalexin	-21.893	0.074	100	336	100
Cefadroxyl	-20.637	0.139	100	170	—
Cefuroxime	-21.755	0.074	100	334	—
Cefoperazone	-11.110	0.316	100	44	50

¹MRL = maximum residue limits.

Table 4. Logistic regression model variables and detection limits of the β -lactams obtained using Eclipse 100 (ZEU-Immunotec SL, Zaragoza, Spain) in goat's milk considering a binary response variable

Antimicrobial	Intercept (a)	Slope (b)	Concordance coefficients (%)	Detection limits ($\mu\text{g/kg}$)	MRL ¹ ($\mu\text{g/kg}$)
Penicillin G	-28.753	11.504	100	3	4
Ampicillin	-50.941	11.343	83.3	5	4
Amoxicillin	-50.941	11.343	83.3	5	4
Cloxacillin	-48.008	1.224	66.3	42	30
Dicloxacillin	-30.280	1.215	100	27	30
Oxacillin	-31.252	3.103	98.4	11	30
Cefalexin	-38.231	0.427	80	96	100
Cefadroxyl	-21.000	0.420	100	57	—
Cefuroxime	-38.043	0.424	80	97	—
Cefoperazone	-38.043	0.424	80	97	50

¹MRL = maximum residue limits.

false-positive results, and a high SCC was correlated with a false-positive result. Molina et al. (2003) determined 7 β -lactams at EU MRL in sheep's milk and only penicillin G residues below the MRL were detected. Our results indicate that the BRT is accurate at detecting β -lactam residues in goat's milk at or below EU MRL, with the exception of cefalexin (Table 3 and Figure 2). The other 7 β -lactams with established MRL were detected at or below this limit. The amoxicillin DL (4 $\mu\text{g/kg}$) coincided with the MRL, whereas DL of the 6 remaining antibiotics were below the corresponding MRL: penicillin G (2 $\mu\text{g/kg}$), ampicillin (3 $\mu\text{g/kg}$), cloxacillin (27 $\mu\text{g/kg}$), dicloxacillin (8 $\mu\text{g/kg}$), oxacillin (8 $\mu\text{g/kg}$), and cefoperazone (44 $\mu\text{g/kg}$). Only the DL of cefalexin (336 $\mu\text{g/kg}$) was higher than the MRL. According to these results and contrary to the results of the Delvotest in sheep's milk reported by Molina et al. (2003), the BRT is likely to be better at detecting β -lactam residues at or below the DL in goat's milk than in sheep's milk. Thus, in our study the BRT detected 7 β -lactams at or below the MRL and only its DL for cefalexin was higher than the official limit, whereas Molina et al. (2003) found that the BRT in sheep's milk was only capable of detecting penicillin G

at levels below the EU MRL. For the other 6 β -lactams studied by these authors, the levels detected were above the EU MRL; 3 being close to the MRL (amoxicillin, ampicillin, and ceftiofur) and the remaining 3 well beyond the maximum limit (cloxacillin, cephalixin, and cefoperazone).

The results obtained for the Eclipse 100 test indicate that half the β -lactams studied were detected at levels below the MRL and the other half were close to, but above, this level (Table 4 and Figure 3). Thus, DL below the MRL were recorded for penicillin G (3 $\mu\text{g/kg}$), dicloxacillin (27 $\mu\text{g/kg}$), oxacillin (11 $\mu\text{g/kg}$), and cefalexin (96 $\mu\text{g/kg}$) and DL just above the MRL were observed for ampicillin (5 $\mu\text{g/kg}$), amoxicillin (5 $\mu\text{g/kg}$), and cloxacillin (42 $\mu\text{g/kg}$). The DL determined for cefoperazone (97 $\mu\text{g/kg}$) was double the MRL. The Eclipse test was developed for use in cow and sheep's milk. Montero et al. (2005) used this test in sheep's milk to establish the DL of 6 β -lactams with defined MRL and only oxacillin (28 $\mu\text{g/kg}$) was detected at a level below the limit. Penicillin G (5 $\mu\text{g/kg}$) and cefalexin (115 $\mu\text{g/kg}$) were detected just beyond the limit and the DL established for amoxicillin (7 $\mu\text{g/kg}$), cloxacillin (68 $\mu\text{g/kg}$), and cefoperazone (110 $\mu\text{g/kg}$) were almost

Table 5. Logistic regression model variables and detection limits of the β -lactams obtained using the Copan Milk Test (Copan Italia SpA, Brescia, Italy) in goat's milk considering a binary response variable

Antimicrobial	Intercept (a)	Slope (b)	Concordance coefficients (%)	Detection limits ($\mu\text{g/kg}$)	MRL ¹ ($\mu\text{g/kg}$)
Penicillin G	-16.946	11.270	100	2	4
Ampicillin	-16.946	11.270	100	2	4
Amoxicillin	-28.753	11.504	100	3	4
Cloxacillin	-30.280	1.215	100	27	30
Dicloxacillin	-29.934	2.924	98.6	11	30
Oxacillin	-20.164	3.216	100	7	30
Cefalexin	-12.970	0.430	100	37	100
Cefadroxyl	-21.000	0.420	100	57	—
Cefuroxime	-11.110	0.316	100	44	—
Cefoperazone	-11.110	0.316	100	44	50

¹MRL = maximum residue limits.

Figure 1. Detection pattern obtained for Delvotest MSC (DSM Food Specialties, Delft, the Netherlands) used to detect residues of the 8 β -lactams. Detection limits are indicated as n-fold the EU maximum residue limits expressed in $\mu\text{g/kg}$ (inner circles $\geq 10\times$; middle circles $1\times$; outer circles $0.1\times$).

Figure 3. Detection pattern obtained for Eclipse 100 (ZEU-Immunotec SL, Zaragoza, Spain) used to detect residues of 8 β -lactams. Detection limits are indicated as n-fold the EU maximum residue limits expressed in $\mu\text{g/kg}$ (inner circles $\geq 10\times$; middle circles $1\times$; outer circles $0.1\times$).

twice the official limits. The variation observed here in cloxacillin residue detection (agreement was 66.3%) should be noted, because cloxacillin is widely used for dry treatment in cows as well as goats and sheep. When used to test sheep's milk, samples are diluted to avoid the effect of excess fat. We did not dilute our samples because the fat content of goat's milk is relatively lower. The Eclipse test was the least sensitive for detecting β -lactam residues in goat's milk and would need to be improved for AR screening, as suggested by Montero et al. (2005) for sheep's milk.

Contrary to the other ARST examined, there are no published data on the use of the CMT to test the milk of small ruminants. Because this test was visually read, we checked to ensure that there was agreement between the 2 involved technicians and the agreement was complete (kappa coefficient = 1). Using this test to determine the 8 β -lactams with available MLR, residue levels were always below the MRL (Table 5 and Figure 4). Test sensitivity was high, because cloxacillin and cefoperazone detection limits were close to MRL but the other DL were found to be lower than the MRL,

Figure 2. Detection pattern obtained for BRT AiM (AiM-Analytik in Milch Produktions-und Vertriebs GmbH, München, Germany) used to detect residues of 8 β -lactams. Detection limits are indicated as n-fold the EU maximum residue limits expressed in $\mu\text{g/kg}$ (inner circles $\geq 10\times$; middle circles $1\times$; outer circles $0.1\times$).

Figure 4. Detection pattern obtained for the Copan Milk Test (CMT; Copan Italia SpA, Brescia, Italy) used to detect residues of 8 β -lactams. Detection limits are indicated as n-fold the EU maximum residue limits expressed in $\mu\text{g/kg}$ (inner circles $\geq 10\times$; middle circles $1\times$; outer circles $0.1\times$).

and more than twice this limit for dicloxacillin, oxacillin and cefalexin. Similar results have been reported by Le Breton et al. (2007) for the β -lactams penicillin G, cloxacillin, and cefalexin in cow's milk. This high sensitivity has the benefit of guaranteeing the safety of milk for consumers, but will have the drawback of giving rise to many false positives with the consequent economic losses for producers.

CONCLUSIONS

Most of tests used were able to detect β -lactam residues at levels $1 \times$ the EU stipulated MRL or below. The Delvotest and CMT were the tests that best fulfilled IDF criteria for the ideal AR test. Despite one of the tests being unable to detect levels of 3 of the antimicrobials below the MRL (ampicillin, amoxicillin, and cloxacillin), our results indicate that under the analytical conditions specified by the IDF, the screening methods examined here for detecting β -lactam residues can be reliably used in goat's milk.

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