Performance of current microbial tests for screening antibiotics in sheep and goat milk

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ABSTRACT

The detection capability (CCβ) of some microbial screening tests (BRT MRL, Delvotest MCS SP-NT, Delvotest MCS Accelerator and Eclipse 100) currently available was calculated in accordance with Commission Decision 657/2002/EC. The CCβ was at or below the maximum residue limit (MRL) for most β-lactams assessed and other non-β-lactam drugs such as neomycin, tylosin, sulfadiazine and sulfadimethoxine. However, the tests were less sensitive in the detection of most non-β-lactam drugs such as quinolones and tetracyclines at safety levels. When individual sheep milk samples free of antibiotics were analysed, an elevated somatic cell count (SCC) was related to the occurrence of non-compliant results in all the methods assessed. In order to guarantee the safety of milk and dairy products from small ruminants, the periodical implementation of screening tests more sensitive towards non-β-lactam drugs would be convenient.

1. Introduction

Microbial inhibitor tests are routinely applied for screening antibiotics in raw milk as they are relatively inexpensive, user-friendly, and have a high sample throughput.

Most current microbial screening tests were initially developed to detect β-lactams in cow milk and are based on the inhibition of *Geobacillus stearotermophilus* var. *calidolactis* being highly sensitive to these substances. Several studies on the microbial test sensitivity using sheep milk have been carried out in the last two decades (Althaus, Torres, Montero, Balasch & Molina, 2003a; Molina, Althaus, Molina & Fernández, 2003), while very few studies have been undertaken in goat milk (Sierra et al., 2009a,b), demonstrating that these tests are able to detect β-lactams at or below the Maximum Residue Limits (MRLs) established by European legislation (EC, 2010), but cannot suitably detect other veterinary drugs.

Therefore, modifications such as the addition of chelating agents into the culture medium to enhance the detection of tetracyclines, respectively, of antifolates to improve sulphonamide detection have been proposed (Langeveld, Beukers, Bommele, & Stark, 2005), and consequently manufacturers have improved some performance characteristics of microbial screening tests in new versions now available.

Moreover, sheep and goat milk is characterised by a higher fat and protein content than cow milk (Park, Juarez, Ramos & Haenlein, 2007), an elevated natural inhibitor content (e.g. immunoglobulins, lactoferrin, or lysozyme) (Crosson, Thomas, & Rossi, 2010) and a higher somatic cell count (SCC) even in the absence of intra-mammary infections (Medhid, Díaz, Martí, Vidal & Peris, 2013), potentially interfering in the microbial inhibitor test response.

Sheep and goat milk production is mainly destined for the elaboration of dairy products. Antibiotic residues in milk may partially or totally inhibit fermentation processes in cheese and yogurt production (Packham, Broome, Limsowtin, & Roginski, 2001). Moreover, consumer safety may be compromised by the presence of these residues in dairy products (Oliver, Murinda & Jarayao, 2011). Thus, the aim of this study was to assess the performance of new versions of some microbial screening tests to detect antimicrobial residues in sheep and goat milk according to European Commission Decision nº 657/2002 (EC, 2002).

2. Material and methods

2.1. Microbial inhibitor tests: The screening tests used were the BRT MRL (Analytik in Milch Produktions-und Vertriebs-GmbH. Munich, Germany), Delvotest MCS SP-NT (DSM Food Specialties. Delft, the Netherlands), Delvotest MCS Accelerator (DSM Food Specialties), and Eclipse 100 (Zeu-Inmunotec. Zaragoza, Spain). All tests were conducted according to each manufacturer's instructions.

2.2. Milk samples: Antibiotic-free milk samples were obtained from the experimental flocks of Manchega sheep of Universidad de Castilla-La Mancha (Albacete, Spain), and Murciano-Granadina goats of Universitat Politècnica de València (Valencia, Spain). Animals had a good health status and had not received any veterinary drugs, neither before nor during the experimental period, nor was medicated feed used in their diet. All milk samples were analysed for gross composition (MilkoScan 6000, Foss. Hillerød, Denmark), somatic cell count (Fossomatic 5000, Foss), total bacterial count (Bactoscan FC, Foss), and pH value (pHmeter, Crison, Barcelona, Spain).

2.3. Antimicrobials and spiked milk samples: A total of 37 substances was investigated: amoxicillin, ampicillin, benzylpenicillin, cloxacillin, dicloxacillin, nafcillin, oxacillin, cefalonium, cefapirin, cefazolin, cefoperazone, cefquinome, ceftiofur, cephalexin, chlortetracycline, ciprofloxacin, colistin, enrofloxacin, erythromycin, gentamicin, lincomicin, marbofloxacin, neomycin, oxytetracycline, streptomycin, sulfadiazine, sulfadimethoxine, sulfametazine, tetracycline, trimethoprim, and tylosin were provided by Sigma-Aldrich Química, S.A. (Madrid, Spain). Desfuroylceftiofur was supplied by Toronto Research Chemicals, Inc. (Toronto, Canada) and the 4-epimers of tetracyclines were furnished by Acros Organics (Geel, Belgium). Finally, desacetylcefapirin and cefacetrile, not commercially available, were kindly provided by Fatro S.p.A. (Bologna, Italy) and ACS Dobfar, S.p.A. (Milan, Italy), respectively. Spiked milk samples were prepared following International Dairy Federation recommendations (ISO/IDF, 2003), and tested by the microbial screening tests immediately after spiking.

2.4. Detection capability (CCβ): Test detection capability (CCβ) was investigated following the "Guidelines for the validation of screening methods for residues of veterinary medicines" proposed by Community Reference Laboratories for residues (CRLs, 2010), supplementing Commission Decision nº 657/2002 (EC, 2002).

Antimicrobial-free milk samples were spiked individually with different substances at 0.5·MRL, 0.75·MRL, and 1·MRL equivalent drug concentration, and analysed 20, 40 or 60 times, respectively, by the different microbial tests.

2.5. Interferences related to milk matrix constituents: Antibiotic-free milk samples were obtained on a two-week basis along the entire milking period (sheep: n=250, sampling days 30 to 180 post-partum; goats: n=350, sampling days 15 to 200 post-partum) and analysed simultaneously, in three replicates, by the four tests. The effect of the milk matrix constituents on the test response was investigated using the stepwise option of the logistic procedure of the SAS software, according to the following model:

L_{ij}= logit $[P_i]$ = $\beta_0 + \beta_1[SL] + \beta_2[pH] + \beta_3[F] + \beta_4[P] + \beta_5[L] + \beta_6[TS] + \beta_7[logSCC] + \beta_8[logBC] + \varepsilon_{ij}$ (Eq. 1)

where: $L_{ij=}$ logistic model; $[P_i]$= probability for the response category (positive/negative); $\beta_{0=}$ intercept; $\beta_{i=}$ estimate parameters for the model; [SL]= lactation stage effect (day); [pH]= pH effect; [F]= fat content effect; [P]= protein content effect; [L]= lactose content effect; [TS]= total solids content effect; [logSCC]= somatic cell count effect; [logBC]= bacterial count effect; $\varepsilon_{ij=}$ residual error.

3. Results and discussion

3.1. Detection capability (CCβ): The CCβ of the BRT MRL, Delvotest SP-NT, Delvotest DA, and Eclipse 100 tests in sheep and goat milk were shown in Table 1. In general, microbiological tests detected high frequencies of β-lactam antibiotics in milk from sheep (70.6 %: BRT MRL, and 88.2 %: Delvotest SP-NT, Delvotest DA, and Eclipse 100) and goats (76.4 %: BRT MRL, and 82.3 %: Delvotest SP-NT; Delvotest DA, and Eclipse 100). Only cefquinome and cefoperazone could not be detected by any test at MRL equivalent antibiotic concentration.

For non-β-lactam drugs, the microbiological tests could not detected residues of quinolones, tetracyclines, streptomycin, lincomicin, sulfametazine, colistin and trimethoprim at safety levels. Conversely, neomycin, tylosin sulfadiazine and sulfadimetoxine were detected at or below regulatory limits (Table 1). Moreover, the BRT MRL test was also able to detect gentamicin and erythromycin at their MRLs.

3.2. Interferences related to the milk matrix effect

Individual milk, free of antimicrobials, presented a wide range of variation for all milk quality parameters considered. When individual sheep milk samples were analysed an elevate percentage of non-compliant results were obtained (BRT MRL: 4.8 %, Delvotest SP-NT: 8.0 %, Delvotest DA: 10 % and Eclipse 100: 9.6 %). Applying logistic regression analysis, an increase in SCC was associated with an elevation in the predicted likelihood of positive outcomes in all cases (Figure 1), the BRT MRL test response being the less affected by this parameter. These results were in agreement with those obtained by Cullor et al. (1992) and Althaus et al. (2003b) using individual milk samples from cows and sheep, respectively. Instead, the percentage of non-compliant results in goat milk were lower in all cases (BRT MRL: 1.4 %, Delvotest SP-NT: 4.3 %, Delvotest DA: 3.1 % and Eclipse 100: 0.6 %), and therefore, logistic procedure was not performed.

4. Conclusions

Microbial inhibitor tests are efficient to detect β-lactams and other non-beta-lactam drugs such as neomycin, tylosin, sulfadiazine and sulfadimethoxine in raw milk from small ruminants. However, in spite of the improvements made in these tests in the last decade, they continue to be inefficient for the detection of other drugs, such as quinolones and tetracyclines, at safety levels. Therefore, the periodic use of more sensitive tests towards these substances would be convenient to widen the detection range in screening and guarantee the quality of milk and dairy products from small ruminants.

5. Acknowledgements

This research forms part of the Project AGL2009-11524 financed by the Ministerio de Ciencia e Innovación (Madrid, Spain).

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Table 1. Detection capability (CCβ) of microbial screening tests for the detection of antimicrobials in sheep and goat milk.

Antimicrobial	MRL[a] (µg·Kg^{-1})	CCβ[b] sheep/goat (µg·Kg^{-1})			
		BRT MRL	Delvotest SP-NT	Delvotest DA	Eclipse 100
β-lactams					
Amoxicillin	4	4/3	3/4	3/4	3/4
Ampicillin	4	3/2	3/2	3/3	4/4
Benzylpenicillin	4	3/2	3/2	3/2	4/2
Cefacetrile	125	≤63/≤63	≤63/≤63	≤63/≤63	≤63/≤63
Cefalonium	20	20/15	≤10/15	20/15	20/15
Cefapirin	60	≤30/≤30	≤30/≤30	≤30/≤30	≤30/≤30
Deacetylcefapirin	*	≤30/≤30	≤30/≤30	≤30/≤30	≤30/≤30
Cefazolin	50	≤25/≤25	≤25/≤25	≤25/≤25	≤25/≤25
Cefoperazone	50	>50/>50	>50>50	>50/>50	>50/>50
Cefquinome	20	>20/>20	>20/>20	>20/>20	>20/>20
Ceftiofur	100	>100/>100	100/>100	100/>100	100/>100
Desfuroylceftiofur	*	>100/100	75/100	100/100	100
Cephalexin	100	>100/>100	≤50/75	≤50/≤50	≤50/≤50
Cloxacillin	30	23/23	≤15/23	≤15/23	23/23
Dicloxacillin	30	≤15/≤15	≤15/≤15	≤15/≤15	23/≤15
Nafcillin	30	≤15/≤15	≤15/≤15	≤15/≤15	≤15/≤15
Oxacillin	30	≤15/≤15	≤15/≤15	≤15/≤15	≤15/≤15
Aminoglycosides					
Gentamicin	100	100/100	>100/>100	>100/>100	>100/>100
Neomycin	1,500	≤750/≤750	≤750/≤750	≤750/≤750	>1,500/>1,500
Streptomycin	200	>200/>200	>200/>200	>200/>200	>200/>200
Macrolides					
Erythromycin	40	40/40	>40/>40	>40/>40	>40/>40
Lincomicin	150	>150/>150	>150/>150	>150/>150	>150/>150
Tylosin	50	≤25/50	≤25/≤25	≤25/50	≤25/50
Sulphonamides					
Sulfadiazine	100	>100/>100	75/≤50	75/75	75/≤50
Sulfadimethoxine	100	≤50/≤50	≤50/≤50	75/≤50	100/100
Sulfametazine	100	>100/>100	>100/>100	>100/>100	>100/>100

[a]MRL: Maximum Residue Limit (µg·Kg^{-1}) established by EC Regulation nº 37/2010 (EC, 2010); [b]CCβ: Detection Capability (lower antimicrobial concentration which produces at least 95 % positive results); *: marker residue. MRL not established.

Figure 1. Effect of the somatic cell count (SCC) on the false non-compliant outcomes of microbial screening tests using sheep milk.