PAPER

Survey on antimicrobial residues in raw milk and antimicrobial use in dairy farms in the Emilia-Romagna region, Italy

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Abstract

This survey investigated the antimicrobials most commonly used in dairy herds and antimicrobial residues most frequently detected in milk to evaluate the suitability of rapid screening tests to determine antimicrobial residues in milk. The investigation was carried out in 45 dairy herds consulting the farm administration records and in a national dairy industry collecting milk from almost all the dairy farms studied. Data were recorded on: i) treatments with drugs containing antimicrobials during the 12 months prior to the visit; ii) antimicrobial active substances present in the drugs; iii) data from routine controls to detect antimicrobial residues (52,771 samples). The antimicrobial classes most commonly used were penicillins, cephalosporins, fluoro-quinolones, macrolides, sulphonamides, tetracyclines, aminoglycosides and lyncosamides; the most frequently used antimicrobial not belonging to any of the previous groups was riphaximin. Sixty-four samples collected from milk trucks yielded antimicrobial residues exceeding the detection limit of the screening test used: sulphonamide residues were the most prevalent (3.4%), followed by tetracycline (0.3%) and penicillins and cephalosporins (0.03%). The antimicrobial classes most commonly used on dairy farms are the same as the residues most frequently detected in milk. The association of several commercially available rapid test kits proved satisfactory for determination of the veterinary antimicrobial drugs most used on dairy farms but at least five kits are required.

Introduction

Antimicrobial agents are widely used in dairy cattle management for prophylactic and therapeutic purposes. Although the use of these drugs has significantly improved the health and production efficiency of food-producing animals (Alomirah *et al*., 2007), incorrect use, improper administration or mistakes by farmers and veterinarians in observing the withdrawal time for treated animals can result in antimicrobial residues in milk and dairy products. Antimicrobial residues are currently the most common inhibitory substances detected in milk, and may have undesirable effects on milk quality, milk technological properties, dairy product quality, and last but not least human health (Ortelli *et al*., 2009, Piñero *et al*., 2012, Zhan *et al*., 2012).

The importance of monitoring antimicrobial residues in milk is emphasized by the role of milk and dairy products in human nutrition (Ortelli *et al*., 2009). The European Union (EU) has established safe maximum residue limits (MRLs) for pharmacologically active substances in foodstuffs of animal origin (European Commission, 2010). Several chromatographic confirmatory methods, which must comply with Commission Decision 657/2002/EC, have been described for the determination of antimicrobials in food. However, all routine monitoring tests on raw milk in bulk tank trucks should be completed as soon as possible (usually in less than 3 h) before the milk is unloaded for processing. Monitoring a large number of milk samples for the presence of residues requires rapid low cost screening methods. Dairy plant monitoring is primarily performed using microbiological and immunological screening methods (Bilandžić *et al*., 2011; Zhan *et al*., 2012) but these techniques present some drawbacks. Microbiological screening methods may have an analytical sensitivity lower than the MRLs allowed by law, while immunological screening methods require one test kit for each family of antimicrobials and the sensitivity depends on the cross-reactivity of each compound resulting in very different detection limits within the same drug family (Ortelli *et al*., 2009).

The European Regulation No. 178/2002/EU (European Commission, 2002) states that in EU the food business operators have the pri-

mary legal responsibility for ensuring food safety, and it is generally recognised that producers are in the best position to devise methods for warranting the food safety (Vragović *et al*., 2011). In dairy plants, it may be difficult to implement a control programme to avoid residues of veterinary antimicrobials in milk due to the large number of different compounds and formulations commercially available. Knowledge of the antimicrobial substances most frequently used in dairy farms will serve to choose appropriate screening tests to detect antimicrobial residues in milk. The aim of the survey was therefore: i) to monitor the antimicrobial agents most frequently used in dairy farms in the Emilia-Romagna region and to evaluate the ability of the screening kits used for veterinary drug residue determination in raw milk to detect residues of these antimicrobial agent; ii) to evaluate the agreement of the data collected on the most frequently used antimicrobials with the data on the most common antimicrobial residues tested in milk by a national dairy industry; iii) to evaluate the availability of screening test kits able to detect potential residues of the most frequently used antimicrobial drugs.

Materials and methods

The survey was conducted in 45 dairy farms located in the Emilia-Romagna region in northern Italy and data were collected between January and December 2012. Milk was primarily produced for industry (destined for pas-

teurization) and secondly for *Parmigiano Reggiano* cheese production; farmers usually belong to a cooperative. The dairy farms surveyed were chosen based on herd size and farm typology. The farms had an average of 98 milking cows (from a minimum of 16 to a maximum of 300) and the daily milk production was estimated to be from 150 L to 6400 L.

With the farmers' consent, information was collected and checked in the farm register of veterinary drugs during a visit to each farm: the number of antimicrobial administrations in the previous 12 months was verified to record the inventory of veterinary antimicrobials used. For each farm and for each antimicrobial agent used, the frequency (F) was quantified as:

F=number of animals treated with the antimicrobial agent*total treatment days for each treated animal

The total incidence of the use of antimicrobial agents (F total) was calculated as sum of the F of each antimicrobial agent.

To determine the most frequent antimicrobial residues detected in milk, we used the data of routine controls performed in 2012 by a national dairy industry on milk trucks before unloading milk; the dairy industry in question collects the milk from almost all the dairy farms studied. The number of samples, screening test kits used and detection limit of antimicrobial substances are listed in Table 1. To assess the ability of the commercial screening test kits to detect the most commonly used antimicrobials, we considered the kits most frequently used in Italy. Test sensitivity data were collected from the documents published in the literature or by manufacturers (Table 1).

Results

Our study showed that the dairy farms investigated used a total of 43 veterinary drugs with 32 active substances recorded in the commercially available formulations. Table 2 summarizes the list of active substances and their frequency of use in the dairy farms. The total frequency of these active substances use was 16,972 (Ftotal=number of treated animals* treatment days); 16 active substances showed F>250 and on the whole a F equal to 15,331, *i.e.*, 90% of the F total. The remaining 16 active substances not included in the main active substances were marbofloxacin, dihydrostreptomycin, neomycin, cephalonium, sulphamerazine, cephapirin, spiramycin, trimethoprim, sulphamethopyrazin, thiamphenicol, cephoperazone, penethamate, benetaminpenicillin, framycetin, sulphapyridine and naphcillin.

The antimicrobial agents used belonged to the following classes: lactams (51.3% of the total use of antimicrobial agents), fluoroquinolones (8.8%), macrolides (4,6%), sulphonamides (3.1%), tetracyclines (2.6%), lyncosamides (2.1%) and amphenicols (0.3%); other antimicrobial agents used not belonging to the previous reported classes were: riphaximin, clavulanic acid and trimethoprim (respectively 12.4, 11.2 and 0.5% of the total antimicrobial agents used).

Table 1. Number of milk samples analysed and sensitivity of different kits for the antimicrobial substances tested during routine controls performed by a national dairy industry in 2012 and maximum residue limits regulated by European Regulation 37/2010/EU.

	N of samples	Tests used	Pharmacologically active substance	Sensitivity charm,° µg/kg	Sensitivity DelvotestSP°, µg/kg	MRL, µg/kg
β lactams	45,677	Charm MRL/ Delvotest SP	Amoxicillin	3 - 4	3-5	4
			Ampicillin	3 - 4	3-5	4
			Cephacetril	8 - 18	20-40	125
			Cephalexin	30 - 60	60-100	100
			Cephalonium	3 - 5	10-25	20
			Cephazolin	12 - 20	no claim	50
			Cephoperazone	5 - 9	60-100	50
			Cephquinome	15 - 20	no claim	20
			Ceftiofur	10 - 20	50-70	100
			Cephuroxime	3 - 5	no claim	nr
			Cephapirin	6 - 10	5-10	60
			Cloxacillin	25 - 35	15-25	30
			Dicloxacillin	20 - 30	10-15	30
			Penicillin G	2 - 3	2.5	4
			Oxacillin	nr	10	30
			Naphcillin	nr	5-10	30
Enrofloxacin	759	Charm Enrofloxacin Test	Enrofloxacin	8		100
Tetracyline	3752	Charm Tetracycline Test	Chlortetracycline	50-100		100
			Oxytetracycline	50-100		100
			Tetracycline	10-30		100
Sulphonamides	1040	Charm Sulfa Test	All substances belonging to the sulphonamide group	100		100
Streptomycin	765	Charm Streptomycin Test	Streptomycin	75		200
			Dihydrostreptomycin	75		200

°Detection limit reported in literature (Bilandžić *et al.*, 2011) or by manufacturers (Charm, 2013; DSM, 2013; Tecnalab, 2013; Zeulab, 2013; Neogen, 2013; Globalcube, 2013); MRL, maximum residue limit; nr, not reported.

A total of 64 samples yielded antimicrobial residues exceeding the detection limit of the screening test used (Table 3). Briefly, sulphonamide residues were most prevalent, present in 36 samples (3.4%), followed by tetracycline in 13 samples (0.3%) and β lactams detected in 15 samples (0.03%). The analysis of commercially available rapid test kits showed that they were able to detect 14 out of 16 antimicrobial substances most frequently used (F>250) in the Emilia-Romagna region, except for clavulanic acid and riphaximin. To determine the antimicrobial agents less used on the dairy farms studied, test kits are commercially available for the determination of 13 out of 16 antimicrobial substances, except for sulphamethopyrazin, benetaminpenicillin and framycetin.

Discussion

Our data on the frequency of antimicrobial use in the dairy farms surveyed partly overlap data reported by the Official Veterinary Service during a monitoring performed in the Emilia-Romagna region (Moro *et al*., 2006), in which β lactams, macrolides, aminoglycosides, polymyxins, suphonamides, amphenicols and tetracyclines were the most used antimicrobial classes in bovine herds. Our study found that penicillins, cephalosporins, fluoroquinolones, macrolides and sulphonamides respectively were the most used classes of antimicrobials. The discrepancies in the two studies depend on their different aims and the consequent different data collection methodology on antimicrobial use: in the study of Moro *et al.* (2006) data on antimicrobial use in both beef and dairy production were collected, instead, in our study only dairy farms were taken into account in order to record the veterinary drugs that could potentially result in antimicrobial residues in milk and milk products. As the most common diseases differ in beef and dairy production, disease management (antimicrobial substances, dosage, associations, route of administration, *etc*.) may also differ. The use of antimicrobials in dairy farms varies in different countries (Hill *et al*., 2009; González *et al*., 2010; Saini *et al*., 2012), and this may depend on the different Regulations in force. Our findings can be roughly compared to the study by Hill *et al*. (2009) in the USA which reported that the most common primary treatments for both lameness and respiratory disease in lactating cows were penicillins, cephalosporins and tetracyclines, whereas cephalosporins were the most common primary treatment for mastitis, closely followed by penicillins and then macrolides. González *et al*. (2010) reported that penicillins, sulphonamides, aminoglycosides and tetracyclines were the most frequently used active substances in Switzerland, whereas use of third and fourth generation cephalosporins, fluoroquinolones and macrolides was low. Cephalosporins, penicillin combinations, and tetracyclines were the most commonly used antimicrobial drug classes on Canadian dairy farms (Saini *et al*., 2012) similar to findings on Dutch dairy farms (Mevius *et al.*, 2009). Generally speaking, β lactams were used on all dairy farms and constituted the highest proportion of antimicrobials with cephalosporins used at a higher rate than penicillins. The comparison of data on the antimicrobials most commonly used in dairy herds with the data collected on the antimicrobial residues most frequently detected in milk in this study showed a good overlap; few data have been reported by the rapid alert system for food and feed (RASFF) on veterinary drug residues in milk and milk products in recent years. Literature reports are also scarce: Bilandžić *et al*. (2011) monitored Croatian veterinary drug residues from 2008 to 2010, reporting a low frequency of positive samples (<1%) belonging to sulphonamides, tetracycline, aminoglycosides and fluoroquinolones. Considering no β lactam or riphaximin residue determination was performed in the Croatian study, there may be a non-negligible overlap with our data both in veterinary antimicrobial drugs used in dairy farms and residues in milk in which sulphonamides, tetracycline, and β lactams

Table 2. List of active substances and frequency of use in the dairy farms studied.

	Usage frequency°	%	Associations with other antibiotics (number)[#]
Antimicrobial agent			
Amoxicillin	2985	17.59	Clavulanic acid (2)
Riphaximin	2109	12.43	Cephacetril (1)
Clavulanic acid	1908	11.24	Amoxicillin (2)
Ampicillin	1296	7.64	
Enrophloxacin	1281	7.55	
Dicloxacillin	963	5.67	Ampicillin (2)
Cephacetrile	855	5.04	Riphaximin (1)
Ceftiofur	849	5.00	
Cephquinome	639	3.77	
Oxytetracycline	453	2.67	
Cloxacillin	423	2.49	
Lyncomycin	372	2.19	Spectinomycin (1)
Spectinomycin	372	2.19	Lyncomycin (1)
Tylosin	312	1.84	
Sulphamethazine	261	1.54	Sulphamerazine (1); Trimethoprim
Penicillin G	253	1.49	Dihydro-streptomycin (3); Naphcillin (1)
Others	1641	9.67	
Total	16,972	100	

°Number of animals treated with the antimicrobial agent x total treatment days for each treatment in the period of observation (1 year); #number of veterinary drugs in which the antimicrobial agents are associated in commercial formulation (data regarding our observations).

Table 3. Number of tested antimicrobial residues exceeding the maximum residue limits permitted in raw milk samples destined for pasteurization collected in the Emilia-Romagna region in 2012.

	Number of positive samples	Percentage of positive samples
Antibiotic detected with rapid method		
β lactams	15	0.03
Enrofloxacin	0	0.00
Tetracyline	13	0.34
Sulphamides	36	3.46
Streptomycin	0	0.00
Total	64	0.12

were detected. However, aminoglycosides were not investigated in the Croatian survey.

The antimicrobial residues in pasteurized milk from Brazil observed by Zanella *et al.* (2010) included aminoglycosides, tetracycline and β lactams, and were similar to our findings, except for the high antimicrobial residues (30.8%) which were, however, below the MRLs permitted in Brazil.

Nowadays, rapid test kits (microbiological or immunoreceptor-based) for the common antimicrobial drug residues in milk are increasingly used and the risk of milk contamination not detected by an available screening test is very limited. No validated test kits are available for several antimicrobial substances but the commercial formulation must be taken in account for evaluating the risk of the presence of residues: clavulanic acid is sold exclusively in association with substances belonging to the β lactams class for which validated test kits are available; riphaximin is not absorbed and is usually used for the veterinary treatment of reproductive system infections even though formulations exist for mastitis and dry-cow therapy and in this last case riphaximin is sold in association with cephacetrile for which validated test kits are commercially available; benetamin-penicillin and framycetin are usually used for intramammary therapy and are sold in association with penethamate for which validated test kits are available.

Conclusions

The association of several commercially available rapid test kits proved satisfactory for determination of the most used veterinary antimicrobials but at least three kits are required. For the determination of antimicrobial substances less frequently used further two test kits are needed. An effective control plan to avoid the presence of veterinary drug residues requires frequent milk monitoring and the use of several test kits making antimicrobial testing expensive and time-consuming. Our results show that the antimicrobial classes most commonly used in dairy farms are the same as those most frequently detected as residues in milk. Therefore knowledge of the most frequently used veterinary drugs and periodic monitoring are required for the dairy industry to develop a targeted and effective control plan to avoid processing milk containing veterinary drug residues. No currently validated screening test kits are commercially available for the five most common antimicrobial agents.

Given the commercial formulation marketed, the risk of antimicrobial residues can be considered low but our results highlight the need to develop more rapid screening kits or to assess the sensitivity of currently available microbiological tests for these antimicrobial substances.

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