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Review

Antibiotics in Dairy Production: Where Is the Problem?

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Abstract: Antibiotics have long been used for the prevention and treatment of common diseases and for prophylactic purposes in dairy animals. However, in recent decades it has become a matter of concern due to the widespread belief that there has been an abuse or misuse of these drugs in animals and that this misuse has led to the presence of residues in derived foods, such as milk and dairy products. Therefore, this review aims to compile the scientific literature published to date on the presence of antibiotic residues in these products worldwide. The focus is on the reasons that lead to their presence in food, on the potential problems caused by residues in the characteristics of dairy products and in their manufacturing process, on the development and spread of antibiotic-resistant bacteria, and on the effects that both residues and resistant bacteria can cause on human and environmental health.

Keywords: dairy animals; antibiotics; antibiotic residues; milk; dairy products; antibiotic resistance; resistant bacteria

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1. Use of Antibiotics in Dairy Animals

Antibiotics are defined as naturally occurring, semi-synthetic or synthetic compounds with antimicrobial activity that can be applied parenterally, orally, or topically. Antibiotics have been used in livestock care for more than 60 years for the prevention and therapy of common pathologies (mastitis, respiratory and foot diseases, etc.) and for prophylactic purposes. The use of antibiotics in animals destined to food production has been estimated to account for 73% of global antibiotic use [1] and 80% in the United States [2]. In Europe, the last joint report from the European Centre for Disease Prevention and Control (ECDC), European Food Safety Authority (EFSA), and European Medicines Agency (EMA) [3] indicates that the overall antibiotic consumption was, for the first time, lower in food-producing animals than in humans during the period covered in the report (2016−2018). The report concludes that the measures taken in Europe, at the state-level, to reduce the use of antibiotics in food-producing animals are being effective.

Tiseo et al. [4] estimated the global trends in antibiotics use in food animals from 2017 to 2030. They concluded that sales are expected to rise 11.5% by 2030. However, this increase is lower than previous estimates (53%) [2] due to recent reports indicating a decline in antibiotic use, particularly in China, the world's largest consumer.

Milk and dairy products are food of great nutritional, social and economic importance, produced all around the world by very diverse production systems and technologies [5]. Mastitis is the most frequent infectious disease in dairy animals, causing important economic losses in the dairy industry, despite the introduction of mastitis control programs over the last 30 years [6]. In United States, 16% of all lactating dairy cows receive antibiotic therapy for clinical mastitis each year [7]. Antibiotics used for its treatment include β-lactams (penicillin, cefapirin, ceftiofur, amoxicillin, hetacillin, and cloxacillin), macrolides (erythromycin), coumarines (novobiocin), and lincosamides (pirlimycin). In addition, during the dry period, cows are treated for existing subclinical mastitis infections and for

prevention [8]. In United States, more than 75% of all dairy cows receive intramammary infusions of prophylactic doses of antibiotics following each lactation, primarily penicillins, cefalosporins, or other β-lactams [7]. Currently, however, due to the growing concerns about antibiotic resistance, selective preventive treatment is being studied and considered worldwide in herds with low levels of contagious mastitis problems [9].

Other infectious diseases common in dairy cows are respiratory and uterine infections and infectious foot disease. Antibiotics used to treat foot infections include sulfonamides, β-lactams, tetracyclines, and lincomycin. Respiratory diseases or metritis are commonly treated with ceftiofur and other β-lactams, tylosin, tilmicosin, florfenicol, tetracyclines, and sulfadimethoxine [10].

On the other hand, the overall increase in milk production by dairy sheep and goats has resulted in an increased use of antimicrobials to treat mastitis and other diseases in these animals as well [6,11,12]. However, the availability of drugs registered for the use in lactating dairy sheep and goats is quite limited, leading to off-label use of some antibiotics by veterinarians and farmers [13].

2. Antibiotic Residues in Milk and Dairy Products

2.1. Origin of Antibiotic Residues in Milk

Residues, as defined by the European Union (EU) and the Centre for Veterinary Medicine, an agency under the Food and Drug Administration (FDA/CVM) in the United States, are "pharmacologically active substances (whether active principles, recipients or degradation products) and their metabolites which remain in foodstuffs obtained from animals to which the drugs in question have been administered" [14]. To ensure food safety for consumers, several regulatory authorities around the world, including the EFSA, FDA, and Codex Alimentarius, established tolerance levels of antibiotic residues (Maximum Residual Limit, MRL) in milk and other foodstuffs for consumer protection [15]. Table 1 shows the antibiotics used in human medicine and/or dairy animals and their MRL values as established by the European regulation.

Numerous factors influence the concentration of residues in milk, including the characteristics and health of the animal, the amount and type of the administered antibiotic, the method of antibiotics administration, the quantity of milk produced, etc.

Antimicrobials should be applied under veterinary prescription using authorized products and respecting the dose, the routes of administration, and withdrawal periods recommended by the manufacturers [13]. Once administrated to the animal, a big part of the antibiotic is metabolized for the purpose of detoxification and excretion. In general, most of the parent product and its metabolites are excreted in urine and, to a lesser extent, via faeces. However, part of the drug may persist for a period in the animal and can be found in the milk and meat [16]. In addition, for treating mastitis, antibiotics are generally administered by the intramammary route. By this route, the active antibiotic reaches high concentrations at the infection site, being more effective at lower doses [17]. However, the administered drug can be easily transferred from the mammary gland to the milk and is, therefore, the main cause of the presence of residues in it [18]. With intramammary application, residues are found for a longer period and in higher concentrations in milk than in cases where antibiotics are applied parenterally [19]. Because of that, it is compulsory to respect the 'withdrawal period'.

Table 1. Antibiotics used in human medicine and/or dairy animals (adapted from [20]).

Chemical Class	Compound	MRL (μg kg^{-1}) *	Primary Use
Aminoglycosides	Gentamycin	100	All animals, humans
	Kanamycin	150	Dogs, pigs, cattle, horses
	Neomycin	1500	All animals
	Spectinomycin	500	Dogs, pigs, cattle, horses
	Streptomycin	200	Obsolete
Quinolones	Enrofloxacin	100	All animals
	Ciprofloxacin	100	Humans
	Difloxacin	Not for use in animals from which milk is produced for human consumption	
	Danofloxacin	30	
	Marbofloxacin	75	All animals
	Flumequine	50	Humans
	Oxolinic acid	Not for use in animals from which milk is produced for human consumption	
β-Lactams (penicillins)	Amoxicillin	4	All animals
	Ampicillin	4	All animals
	Benzylpenicillin (Pen G)	4	All animals
	Cloxacillin	30	Cattle
	Dicloxacillin	30	Cattle
	Nafcillin	30	Humans
	Oxacillin	30	Cattle
β-Lactams (Cefalosporins)	Cefalonium	20	Dogs, cats and cattle
	Cefazolin	50	Humans
	Cefoperazone	50	Humans, cattle
	Cefquinome	20	Cattle, pigs
	Cefapirin	60	Cattle, sheep, goat and pigs
	Ceftiofur	100	Cattle, pigs
	Desacetylcefapirin	60	Metabolite of cefapirin
	Desfuroylceftiofur	100	Metabolite of ceftiofur
Macrolides	Tylosin	50	Animals only
	Tilmicosin	50	Sheep, cattle
	Spiramycin	200	All animals
	Neospiramycin	20	Metabolite of spiramycin
	Erythromicyn	40	Humans, cattle, chicken
Sulfonamides	Sulfadiazine	100	Humans
	Sulfadimethoxine	100	Cattle, pigs, chicken
	Sulfadimidine	100	Cattle, sheep, chicken
	Sulfamerazine	100	Humans and animals
	Sulfamethoxazole	100	Human
	Sulfamonomethoxine	100	Humans
	Sulfatiazole	100	Humans
	Trimethoprim	100	In combination with sulfonamides
Tetracyclines	Chlorotetracycline	100	Cattle, pigs
	Oxytetracycline	100	Humans, cattle, sheep, pigs
	Tetracycline	100	Humans, cattle, sheep, pigs
	Doxyicycline	Not for use in animals from which milk is produced for human consumption	
Lincosamides	Lincomycin	150	Pigs, cats, dogs, cattle
Amphenicols	Tiamphenicol	50	All animals
	Florfenicol	Not for use in animals from which milk is produced for human consumption	
	Florfenicol amine	Not for use in animals from which milk is produced for human consumption	
Nitroimidazoles	Dimetridazole	Prohibited	
	Ronidazole	Prohibited	
	Metronidazole	Prohibited	

* According to European regulation REG. 37/2010/CE.

As defined by Article 4 of the European Regulation (EU) 2019/6 [21], 'withdrawal period' is the minimum period between the last administration of a veterinary medicinal product to an animal and the production of foodstuffs from that animal. Under normal conditions of use, this period is necessary to ensure that such foodstuffs do not contain residues in quantities harmful to public health. This period has been determined, for different antibiotics, using scientific data and has to be provided by the supplier in the summary of the drug characteristics. For milk-producing animals, withdrawal periods have been established, in most cases, for cattle. For other dairy animals, like sheep and goats, the period has been determined for the most commonly used antibiotics. For antibiotics for which it is not provided, the European Regulation, e.g., sets some criteria to be applied by the veterinarian to calculate the minimum withdrawal period to be set, taking into consideration, among others, the time established for other dairy animals [21].

2.2. Antibiotic Residues in Commercial Cow's Milk Worldwide

The presence of antibiotics in commercial cow's milk has been known for many years. In early studies [22], it was reported that approximately 12% of the United States' cow milk supply was adulterated with β-lactam antibiotics prior to 1962. In Britain, in 1963, 11% of cow milk samples tested were found to contain penicillin. In 1998, data estimated that 1% of animal products in the United States and Europe contained antibiotic residues at very low levels [22]. In addition, a study published in 2000 [23] indicated that between 1988–1990, milk commercialized in North America contained detectable levels of tetracyclines (up to 80% of analysed samples in some studies), sulfamethazine, and other antibiotics. Nevertheless, the results of these early studies showed considerable variability due, as speculated by Mitchell et al. [22], to regional differences in animal husbandry, treatment, and slaughter practices and reflect the different sampling and test methodologies used.

Sachi et al. [16] reviewed the scientific literature that had antibiotic residues in milk as a research topic in the period between 1960 and 2017. They found 224 articles where antibiotic residues were analysed, quantitatively and qualitatively, in cow milk samples. However, the majority of works (82.14%) were about detection methods in which few samples were analysed and, in the majority, milk was spiked with known amounts of antibiotics in order to optimize the method.

In the same way, Treiber et al. [1] reviewed the scientific literature (from 1999 to 2019) about residues of antibiotics in food from an animal origin, focusing on commercial products. They found 73 studies in which antimicrobial residues were analysed in animal products; among them, 27 studied antimicrobial residues in milk. In relation to the importance of the topic, the number of publications found was relatively small.

Table 2 shows a detailed analysis of the articles cited in the mentioned reviews [1,16] in which data on commercial samples were provided. This table also includes articles found in a new search in which the same criteria as the cited reviews have been applied for the years 2019 to the present. Although the table does not pretend to be an exhaustive compilation of all published works, it reflects quite well the imbalance that exists in relation to the territories for which there are data published in the scientific literature in the last twenty years.

As can be appreciated in Table 2, the territories included in these studies correspond, in a great proportion, to Africa, Central and South America, and Asia. Trebier et al. [1] speculated that it might be because in the EU, United States, and some Asian territories, the legal guidelines regarding antibiotic residues in food are relatively strict and are normally checked by state authorities.

Table 2. Published research works on the detection of antibiotic residues in milk.

Territory	Sample Type	No. of Samples	Year [1]	Detection Method	Antimicrobials	% Positive Samples [2]	Concentration	Ref.
Kenya	Raw cow milk	1600	2001–2002	Two-tube diffusion Penicillinase βL plate assay	βL	13%	>4 µg/kg	[24]
	Cow milk	229	2014–2015	Charm II Blue-Yellow HPLC-UV	SAM TC	31.4% 0%	66.14–8979.51 µg/ kg	[25]
	Cow milk	95	2015	IDEXX SNAP®		7.4% βL, 3.2% TC		[26]
	Cow milk	55	2015–2016	IDEXX SNAP®	βL, SMZ,TC, GM	24% in at least one antibiotic		[27]
	Cow milk	65	2020	HPLC	AO, CO,TC, SMX,TriM	10.8% above MRL 20% detectable residues	6.7, 53.3, 30.6, 5.0, 6.2 µg/L, respectively	[28]
Tanzania	Cow milk	982	1999–2000	Charm AIM	βL, TC, AMG, ML, SAM	36%		[29]
	Raw cow milk	91	2006	Delvotest®		4.5%		[30]
	Raw cow milk	128	2006	IDF Method and Delvotest SP®		7%		[31]
	Raw cow milk From dairy farms	98	2010–2011	Delvotest SP®		83%		[32]
Algeria	Cow milk	194	2013–2014	Delvotest SP-NT® LC-MS/MS	βL, ML, SAM, QN, TC	25% (Delvotest®) 65% detectable		[33]
	Cow milk Goats milk	117 33	2019	Delvotest SP® BetaStar® Combo	βL, TC	12.67% (Delvo) 2.5% (cow βL), 1.7% (cow TC) 6.1% (goat βL), 3.0% (goat TC)		[34]
Nigeria	Cow milk	192	2015	Delvotest T®		9.9%		[35]
Ghana	Cow raw milk	224	2007	Charm Blue-Yellow		3.1%		[36]
Brazil	Pasteurized cow milk	151	2005–2006	SNAP tests ELISA kits	TC, βL, GM, CHA, StM, NM	41.3% 4 positive in CHA	dStM 260 µg/kg NM 69.8–110.2 µg/kg CHA 0.157–0.402 µg/kg	[37]
	Pasteurized cow milk	260	2006–2007	SNAP tests Ridascreen	TC, βL, GM, CHA, StM-dStM, NM	TC 18.5%, βL 3.5% GM 2.3%, CHA 1.5%, StM-dStM 0.4%, NM 17.4%	0.16–9.23 µg/kg, 25.86 µg/kg 60.08–278.42 µg/kg	[38]
	Pasteurized cow milk	299	2009	ELISA kit and LC–APCI–MS/MS QToF	StM, dStM	2 samples (ELISA) 0 LC-MS	213 and 290 µg/kg	[39]
	Pasteurized cow milk	252	2010–2011	Delvotest SP-NT® HPLC-DAD	TC OTC	8% positive 10% dubious	107–2297 µg/L 125–2782 µg/L	[5]
	Pasteurized milk	100	2010–2013	HPLC-UV/Vis	OTC, TC, cTC, dOC	3%	Average, 42.3 µg/kg	[40]
	Raw cow milk	184	2020	LC-MS/MS	βL, SAM, TC, QN, fQN, PY	CO (1), PNG (3) TC (11)	464 µg/L, 0.2–4.0 µg/L 7.1–49.7 µg/L	[41]

Table 2. *Cont.*

Territory	Sample Type	No. of Samples	Year [1]	Detection Method	Antimicrobials	% Positive Samples [2]	Concentration	Ref.
Puerto Rico, Barbados, Jamaica	UHT cow milk	80	1996–1997	Delvotest-P® HPLC-UV	βL	Puerto Rico 0% Barbados 8% Jamaica 10%	APC 1.8–18.4 µg/L CF 15 µg/mL CO 61–358 µg/L PNG 6.6–11.8 µg/L	[42]
Paraguay	Cow milk	450	2015	4Sensor and Gentasensor	GM, βL, StM, CHA, TC	0		[43]
Peru	Cow milk	156	2013	Snap Duo™ Beta-Tetra test		0–4.2%		[44]
Iran	Cow milk	196	2008	Copan test kit	βL,TC, SAM, AMG, ML	40.8%		[45]
	Pasteurized/raw cow milk	251	2009–2013	Copan test kit	βL,TC, SAM, AMG, ML	24.8%		[46]
	Pasteurized cow milk	432	2011–2012	HPLC-UV	TC	1.62%	274–1270 µg/L	[47]
	Cow tank milk	79	2012	HPLC-UV	βL	32.9 %		[48]
	Commercial cow milk	187	2012	Eclipse 100-kit HPLC-UV-vis	TC	19.8%	197–2452 µg/kg	[49]
India	Cow milk	491	2016–2017	DPA test and Charm ROSA	βL, TC, NV, EM, SMA	0.6%, 0.8%, 3.5%, 2.4%, 1%		[50]
	Raw/pasteurized cow milk	128/45	2018–2019	HPLC-DAD	AO, TC	1.7%, 1.2%	67.9 ± 40.9, 11.3 ± 1.5 µg/kg	[51]
	Cow milk	168	2019	MaxSignal (ELISA)	EF, OTC, PNG, SMX	1.7%, 1.2%, 0.6%, 0%	87.9 ± 44.0, 70.7 ± 45.9, 2.2 ± 1.5 µg/mL, nd	[52]
Bangladesh	Local/commercial cow milk	200	2011–2012	MIT, TLC HPLC	AO, TC, CPF	AO 14%, 38% local/commer TC 11%, 23% CPF 8%, 17%	AO 9.84, 56.16 µg/mL	[53]
	Cow milk	100	2019	TLC UHPLC	AO, OTC, StM, GM, CTX	2%, 3.33%, 1.33%, 0.6% 0.6 %	AO 124 µg/mL OTC 61.3 µg/mL	[54]

Table 2. *Cont.*

Territory	Sample Type	No. of Samples	Year [1]	Detection Method	Antimicrobials	% Positive Samples [2]	Concentration	Ref.
Nepal	Cow milk	140	2018	Agar diffusion HPLC	PN, SAM	23%	PN 0–16 µg/kg (2 samples 128, 256 µg/kg) SAM 0–64 µg/kg (in 9 samples 128–256 mg/kg)	[55]
Indonesia	Goat milk	36	2018	Triple bio screening test	TC, ML	TC 2.8%, ML 3.6%		[56]
Turkey	UHT cow milk	60	2005	Ridascreen	CHA, StM, TC	46.8% (CHA 30%)	806, 360, 602 ng/L	[57]
Kosovo	Milk from collection points and farms	1734	2009–2010	Delvotest P® SNAP tests, HPLC	βL, TC, SAM	6.11%	AO, PNG, and CO between 2.1 and 1973 mg/kg	[58]
	Cow milk	1055	2015–2016	Delvotest SP, SNAP test		10%		[59]
Croatia	Cow milk	90	2009	ELISA	StM, TC		0–73.82, 0–4.26 µg/kg	[60]
	Raw cow milk	1259	2008–2010	Delvotest® SP-NT Immunoassay (EIA) HPLC-DAD	PN, CPh, TC, SAM, AMG, ML	0.69%	12 µg/kg PNG 19 µg/kg AO, 1671 µg/kg TC	[61]
Slovenia	Raw cow milk	286	1991–2000	GC-ECD	CHA	1 sample	4.6 µg/ kg	[62]
Spain	Ewes raw milk	2686	2004	Delvotest® SP		1.7% positive, 2.1% "doubtful"	βL or SAM n.d.	[63]
	Ewes raw milk	71,228	2004–2008	Eclipse 100ov		1.36% (2004)–0.30% (2008)		[64]
Poland	Fresh and UHT cow milk	36 and 48	2019	PN ELISA Ridascreen	PN TC	1.15% below MRL 28.57% below MRL	0.040 to 0.804 µg/L 0.450 to 2.520 µg/L	[65]

[1] Year of collection or publication (in case no collection year is given). [2] When a screening test is used, data refers to samples that have an antibiotic concentration above the MRL. When a method that allows quantifying the antibiotic concentration is used, data refers to the proportion of samples in which the antibiotics are in concentration above the detection limit. Antibiotic abbreviations: Aminoglycosides (AMG), amoxicillin (AO), ampicillin (APC), β-lactams (βL), ceftriaxone (CTX), cephalin (CF), cephalosporins (CPh), chloramphenicol (CHA), chlortetracycline (cTC), ciprofloxacin (CPF), cloxacillin (CO), dihydrostreptomycin (dStM), doxycycline (dOC), enrofloxacin (EF), erythromycin (EM), fluoroquinolone (fQN), gentamicin (GM), macrolides (ML), neomycin (NM), novobiocin (NV), oxytetracycline (OTC), penicillins (PN), penicillin G (PNG), pyrimidine (PY), quinolones (QN), streptomycin (StM), sulfamethazine (SMZ), sulfamethoxazole (SMX), sulfonamides (SAM), tetracyclines (TC), trimethoprim (TriM). Other abbreviations: Diodo Array Detector (DAD), Electron Capture Detector (ECD), Microbial Inhibition test (MIT), Maximun Residue Level (MRL), not detected (n.d.), Thin Layer Chromatography (TLC).

In this way, the report elaborated by The National Milk Drug Residue Data Base for the FDA in 2020 [66] collected the results of 4,049,727 tests on cow milk samples for three different antibiotic families (β-lactams, sulfonamides and tetracyclines). In this survey, 539 samples (0.013%) were reported as positive (above MRL) for at least one drug residue. With the exception of five samples that were positive for sulfonamides, the rest were positive for β-lactams. Similar results were found in reports of previous years, indicating an improvement in comparison with the early studies.

In the EU, the last report corresponds to 2019 [67]. For the group of antibacterials (e.g., β-lactams, tetracyclines, macrolides, aminoglycosides, sulfonamides and quinolones), 9555 samples of cow milk were tested, and the number of non-compliant samples were 0.12% (one sample in eleven states). However, three positive samples in chloramphenicol were detected (one sample in three states), although the use of this antibiotic is prohibited for veterinary use. Overall, the percentage of non-compliant samples in 2019 was comparable to the previous 11 years.

Data found in scientific literature referring to European states is very scarce (Table 2). Worth mentioning are the data from Kosovo, which are substantially worse than in other European states. The reason may be the fact that Kosovo is not a member of the EU, so it is not subject to its regulations and controls.

In Asia, China has been one of the world's largest dairy consumers for the last few decades, and food safety issues in the dairy sector have increasingly gained the attention of the Chinese government and the public [4,68]. Lu et al. [69] recently have published a review of the studies carried out in this territory. Because of that, we have not included them in Table 2. The review collects 46 surveillance cross-sectional studies published between 1988 and 2020, providing antibiotic levels for 8788 milk samples. Penicillin, tetracycline, chloramphenicol, and streptomycin are the most frequently tested antibiotics in milk samples. The pooled analysis reveals that 165 of 1701 fresh milk samples (9.7%) and 58 of 1220 sterilized milk samples (4.8%) exceeded the MRL limits. Overall, of the 18 evaluated antibiotics in Lu's work, the three with the highest positive rates are sulfamethoxazole, chloramphenicol, and trimethoprim. Nevertheless, although the antibiotic levels in fresh and sterilized milk fluctuate, they have greatly declined in recent years [69].

Data of other Asiatic territories, published in the scientific literature, are very variable and depend on the territory and, in some cases, even on the region, as happens, for example, in the case of Iran (Table 2).

In Central and South America, most published works refer to the situation of Brazil, where it seems to have improved according to the most recent studies.

In Africa, no MRL values are established, but according to values established in the EU or United States, the proportion of non-compliant samples is high in general (Table 2). This is due, most probably, to the fact that in most African territories there is no control over the distribution of veterinary antibiotics because the access to veterinarian pharmaceuticals is still unregulated [1].

The presence of chloramphenicol residues in some samples all around the world is worth mentioning. Chloramphenicol is a very effective broad-spectrum antibiotic, active against a wide variety of pathogens. However, its clinical use in humans can cause fatal side effects such as bone marrow aplasia, Gray baby syndrome, and aplastic anemia. For this reason, the FAO/WHO Expert Committee for antibiotics proposed zero tolerance for its residues in food in 1969 [62]. In the EU, the use of this drug for animal use has been prohibited since 1994 (Directive 1430/94 (EC 1994) [70]). Even so, it is still detected in some milk samples both in Europe, as mentioned before, and in the rest of the world (Table 2).

2.3. Antibiotic Residues in Sheep and Goats Milk

As can be seen in Table 2, the majority of the published data refers to cow's milk. Very few studies analyze milk from other ruminants, such as goats [34,56] or sheep [63,64]. In Mediterranean states, such as Spain, France, Italy, and Greece, the production of sheep and goat milk is mainly intended for the production of dairy products, such as different type

of cheese and yogurt. For this reason, the milk from these animals is not included in the EFSA's surveillance reports. Nevertheless, some states, such as Spain, control the presence of antibiotic residues prior to its use by methods that detect, at least, β-lactams [13].

Thus, very few scientific works have been found where a screening of a large amount of sheep or goats milk samples was carried out. For example, Yamaki et al. [63] analysed a total of 2686 raw sheep milk samples (of Manchega flocks that supplied milk for PDO Manchego cheese, from Spain). They found 1.7% positive results, although the test used did not allow them to identify which were the antibiotics present in the samples. A later work also carried out in Spain [64] analysed 209 dairy sheep flocks of the Assaf breed over 5 years. They obtained 71,228 records and found that the occurrence of non-compliant samples drastically decreased from 2004 (1.36%) to 2008 (0.30%), probably as a result of effective educational programs.

The two studies where commercial goat milk was analysed [34,56] are from Africa and Asia, and, in both, antibiotic residues were found above the MRL in percentages higher than those of sheep milk (Table 2).

2.4. Transfer of Antibiotic Residues from Milk to Dairy Products

Information on the transfer of antibacterial drugs from milk to dairy products found in the scientific literature is based, in most cases, on experimental processes in which antibiotic-free milk is spiked with known amounts of drugs and the effect of different treatments is analysed. Using this type of approach, it has been proved that the transfer depends on the characteristics of the production process and the treatments it includes, as expected [71].

Besides milk, the most consumed dairy products are yogurt and cheese. Although the process to make yogurt can vary depending on the type, in almost all cases, the production includes a first step of pasteurizing the milk. In general, thermal treatment leads to the degradation of antibiotic residues and, consequently, to a reduction of the residue concentration or bioactivity in the food product [72]. However, the values reported in the literature vary widely depending, among others, on the antibiotic, the matrix, and the applied temperature and time. Regarding the matrix, some authors [73] pointed out that penicillins degrade more in water than in milk under thermal treatment. However, other authors concluded that the results for penicillins and tetracyclines are inconclusive [72]. In any case, the temperature and time of application are the main factors that affect the rate of degradation of antibiotics, and, although their effect varies between antibiotics, in general, the treatments applied to pasteurize milk seem to only slightly reduce the concentration of most antibiotics [74]. For this reason, yogurts made from contaminated milk generally show an equal or slightly lower concentration of antibiotics than the milk used for their production [73,75].

Skimming, carried out usually by centrifugation, is used for producing low-fat dairy products. Hakk et al. [76] showed that the distribution of antibiotics between the fractions of milk is, mainly, based on their lipophilicity. They studied the distribution of penicillin G, sulfadimethoxine, oxytetracycline, and erythromycin between milk fat and skim milk fractions of cow milk and found that more than 90% of these antibiotics remained in the skimmed milk. In other studies, it also was found that tetracyclines remained in the skimmed milk in percentages higher than 80% [75] and that sulfonamides were distributed mostly to the aqueous milk fraction [77]. To the contrary, chloramphenicol seems to be mainly retained in high-fat products, such as butter and sour cream, with lower concentrations in white cheese and whey samples [78].

Hundreds of types of cheese are produced in the world, varying in the origin (animal) of the milk and its treatment (raw or pasteurized, full-fat or skimmed . . .) and the technology applied to produce them. Nevertheless, the majority of the following treatments are applied in the production of a great variety of cheeses: warming, starter culture addition and acidification, rennet addition and coagulation, whey draining, curd pressing, and salting. Among all these steps of cheese production, curd production and whey draining

are crucial in the fate of antibiotic residues, and they are the most studied processes using the aforementioned approach. Thus, some authors have studied the distribution of different antibiotics between curd and whey after spiking them to cow [75,79–82], sheep [83,84], and goats' milk [18,74,85,86].

In general, all these studies conclude that the retention of antibiotics in the curd and in the cheese depends fundamentally on their solubility in water and on their ability to interact with the fat and/or protein fraction [76,85]. Thus, β-lactam antibiotics are mostly transferred to whey due to their water-soluble nature [79,80,85], and, due to that, they are found in very low concentration in experimental [7,81] and commercial cheeses [72]. This phenomenon is important since cheese-making by-products, such as whey, are currently recycled in foodstuff manufacturing and are also used for animal feeding [87].

In addition to β-lactams, tetracyclines are the most-studied antibiotics to this respect, it being demonstrated for experimental cow [75], sheep [83,84], and goats [74] cheese that they are mostly retained in the curd and cheese. Giraldo et al. [85] concluded that, in general, aminoglycosides, quinolones, and tetracyclines seem to have a higher susceptibility to be retained in the cheese curd, as they found a reduction of the antimicrobial activity in the whey, ranging from 84 to 100% for these classes of antibiotics. Tetracyclines were also detected in commercial cheeses from Nigeria [71], Indonesia [74], or Pakistan [75]. Quintanilla et al. [15] described discrepant results in soft cheese made from milk enriched with various antibiotics, which they relate to the high whey content in this type of cheese.

Few studies have been found that describe what happens to the residues retained in the cheese during ripening [74,83,84,86]. These studies showed that, in general, the concentration of residues decreases significantly over time. Thus, β-lactams and erythromycin residues were not detectable after 30 days of Tronchon cheese ripening [74]. Quinolones enrofloxacin and ciprofloxacin seem to be more stable, showing a lower reduction rate along maturation (30–45%) [15,86]. Stability data for oxytetracycline varies between studies. In Tronchon-ripened cheese, a 95% reduction in the content of oxytetracycline was measured [74], whereas in 60- and 90-day-old ripened sheep cheese, only a reduction of 15–19% was observed when compared to 1-day ripened cheeses [83,84]. The reduction in antibiotic content during maturation is most likely due to degradation of the molecule. This process may depend on the ripening conditions, which are different for different types of cheese, which, in turn, would explain the different results found among studies.

2.5. Antibiotic Residues in Commercial Dairy Products

Since MRL values are not established for cheese or other dairy products, the analysis of these products is not included in the surveillance reports. Moreover, in the scientific literature, few works have been found where commercial dairy products were analysed (Table 3). As in the case of milk, most studies are from Africa and Asia, and antibiotic residues were found also in a high proportion of the analysed samples.

Table 3. Published research works on the detection of antibiotic residues in commercial dairy products.

Territory	Sample Type	No. of Samples	Year [1]	Method Detection	Antibiotics	% Positive Samples [2]	Concentration	Ref.
Nigeria	cow milk, goat milk, butterfat, soft cheese, yoghurt	8 of each	2014	HPLC-fluorimeter	TC	All below MRL	3.2 ± 1.8, 4.0 ± 1.1, 2.0 ± 0.8 8.0 ± 3.4, 1.9 ± 0.8 µg/L, respectively	[88]
	fresh milk local cheese fermented milk	328 180 90	2016	Premi® test HPLC	PNG	40.8% 24.4% 62.3%	15.22 ± 0.61 µg/L 8.24 ± 0.50 µg/L 7.6 ± 0.60 µg/L	[89]
Burkina Faso	Raw milk Curd Pasteurized milk Yogurt	29 40 42 90	2014	Microbial test	βL, SAM, TC	59.7% of samples positive for some antibiotic		[90]
Indonesia	Imported cheese (Cheddar, Mozzarella)	51	2015	Ridascreen	TC	13.7%	2.47 µg/L to 11.99 µg/L	[91]
Pakistan	Cheese Yogurt	40, 18	2011	HPLC	PNG, StM, TC		6.2, 4.0, 2.3 µg/L 1.7, 1.4, 1.1 µg/L	[92]

[1] Year of collection or publication (in case no collection year is given). [2] When a screening test is used, data refers to samples that have an antibiotic concentration above the MRL. When a method that allows quantifying the antibiotic concentration is used, data refers to the proportion of samples in which the antibiotics are in concentration above the detection limit. Antibiotic abbreviations: β-lactams (βL), penicillin G (PNG), streptomycin (StM), sulfonamides (SAM), tetracyclines (TC). Other abbreviations: Maximum residue level (MRL).

3. Effect of Antibiotic Residues in Dairy Products Elaboration

The presence of antibiotic residues in milk destined to make fermented dairy products could influence the technological processes, causing decreases in the quality of the final products, and, therefore, could have economic consequences for the dairy sector. The problems that the presence of antibiotics can cause in dairy product elaboration were described long time ago [93] and were summarized as failures in the growth of starter cultures, in the curdling of milk, in cheese ripening, and in acid and flavour production.

Lactic acid bacteria (LAB) help dairy products to gain their own aroma, smell, and texture [94]. Many LAB are used as starter cultures for producing fermented dairy products. In addition, non-starter LAB, coming from the raw material and the environment, contribute to the normal development of dairy product characteristics. Thus, most of the problems caused by antibiotic residues are due to the fact that they inhibit the development of LAB, slightly or completely, and delay the acid production by these bacteria. Lowering the pH is very important, for example, in the cheese-making process because it increases the activity of enzymes and the speed of coagulation, which is important especially in hard and long-matured cheeses [95]. In addition, insufficient pH lowering can cause early fermentation, supported by clostridia or by yeasts, and defects in the sensory characteristics of yogurt [96] and cheese [95].

Marth and Ellickson [94] reviewed the susceptibilities of various cheese and yogurt starter cultures to various antibiotics. They compiled the data on concentrations of antibiotics needed for the partial or complete inhibition of activity of various pure or mixed starter cultures and found differences in susceptibilities among species and in the amounts of antibiotics needed to cause inhibitory effects. In the same way, Cogan [97] analysed the susceptibility to penicillin, cloxacillin, tetracycline, and streptomycin of eight single-strain lactic streptococci, three commercial cheese starters, and six lactic acid bacteria isolated from yogurt. They found that the ranges of the antibiotics causing 50% inhibition of the bacteria were (µg/L): penicillin, 9–200; cloxacillin, 240–2500; tetracycline, 90–600; and streptomycin, 350–13,000. The yogurt isolates were found to be more resistant to streptomycin and more susceptible to penicillin than the cheese starters. All values are well above the MRL established for milk (Table 1).

The consequences of the inhibitory effect on the elaboration process and on the characteristics of the final product were analysed through experimental approaches, in which antibiotic-free milk is spiked with known amounts of antibiotics, and the effect of different treatments is analysed, as described before.

Marth and Ellickson [94] collected the studies carried out up to 1959 and showed that the addition of penicillin to milk for cheddar cheese production caused a delay in acid production in a dose-dependent manner. In addition, from a certain dose (variable depending on the study), the ripened cheeses presented a high pH value and defective sensory characteristics, like pasty body and fermented or yeasty aroma.

Similar approaches were carried out in more recent years to study the effect of residues of different types of antibiotics in the production of cheese [74,83,84,86,95,98,99] and yogurt [100–103]. For instance, antibiotic-free goat milk was spiked individually with seven antibiotics (amoxicillin, benzylpenicillin, cloxacillin, erythromycin, ciprofloxacin, enrofloxacin, and oxytetracycline) at an equivalent concentration of the European Union MRL and was used to make Tronchón mature cheeses [74]. The cheese-making process was unaffected by the presence of most antibiotics evaluated. Only erythromycin and oxytetracycline significantly increased the time required for cheese production because the kinetic of acidification was considerably affected by the presence of these antibiotics, requiring additional time to reach the final pH with respect to the control cheeses. The quality characteristics of the Tronchón cheeses were only slightly affected by antibiotics, with few significant differences in free fatty acids (FFA), which were in lower concentration in cheeses with amoxicillin and cloxacillin, and in the colour and some textural properties of the cheeses. Similarly, Cabizza et al. [83,84] showed that oxytetracycline at the MRL level produced a delay in the acidification of sheep milk, with no effect, in general, on physico-chemical parameters and the gross composition of cheeses. Quintanilla et al. [98] also found that the presence of oxytetracycline in goat raw milk, even at a concentration of up to double the MRL, only slightly affected the pH and some parameters of the ripened cheeses (FFA concentration, luminosity, springiness, and chewiness), without being perceptible by the sensory panel. Similar results were found for β-lactams in Manchego cheese elaboration [99] and for lincomycin at concentration lower than the MRL in a bovine milk cheese-making simulation [95]. To the contrary, the quinolone enrofloxacin does not produce significant changes in any of the technological, compositional, texture, and colour characteristics of Tronchón cheese when compared to the cheeses made with antibiotic-free milk, with the only exception of some compounds of the volatile fraction [86].

Similar results were found in the case of yogurt elaboration. For instance, studies with sheep milk yogurt have observed that the levels of some β-lactams (ampicillin, cephalexin and ceftiofur) close to or below the MRL and penicillin G above MRL could delay coagulation by more than 40 min and cause variations in final composition [100–102]. However, no delays were observed with amoxicillin at any concentration [100]. Enrofloxacin added to goat milk for elaborating yogurt also did not significantly affect the coagulation time and most yogurt properties [103].

4. The Use of Antibiotics and the Emergence of Antibiotic Resistant Bacteria

The greatest threat of the use of antibiotics is the emergence and spread of antibiotic resistance (AR) in pathogenic bacteria. Acquired resistance to certain antimicrobials is widespread to such an extent that their efficacy in the treatment of certain life-threatening infections is already compromised [104]. The selective pressure imposed by the use of antimicrobial agents plays a key role in the emergence of resistant bacteria (ARB). In a bacterial population exposed to antimicrobial agents, some are likely to develop resistance to them and, under selective pressure, may pass on their resistance genes to other members of the population [105]. Thus, the presence of antibiotic residues throughout the food chain can cause the development of transferable AR not only in pathogens, but even in commensal bacteria, including LAB [106,107]. Several reports indicate that fermented foods, including dairy products, could be considered as reservoirs of ARB. It has been reported that LAB and *Staphylococcus* sp. were the main AR gene (ARG) carriers in dairy products [108]. LAB isolated from traditional dairy products belong to different genera, such as *Lactococcus* sp., *Lactobacillus* sp., *Enterococcus* sp., *Leuconostoc* sp., and *Streptococcus* sp. Among LAB, enterococci have been the subject of many studies regarding AR due to

the importance of some of them as opportunistic pathogens involved in serious infectious diseases in humans. Some studies have reported the detection of AR and virulence factors of enterococci in foods, including cheeses [109–111].

In the last twenty years, an overwhelming number of articles and reviews have been published describing the antibiotic resistance profile of bacteria, especially LAB, isolated from traditional fermented food, including dairy products. Therefore, it is beyond the scope of this review to collect all the published information on this topic (for that purpose, see, e.g., [106,112–114]).

Surprisingly, the relationship between the use of antibiotics in livestock and the presence of ARB in food has only been established indirectly and remains a contentious subject for study, which does not yield conclusive results [115,116]. This may be due, in part, to the lack of adequate models to study this relationship and because there is a poor understanding of the complex processes that lead to the emergence and spread of AR [115].

Aarestrup [117] collected several experimental and epidemiological studies and ecological observations showing that there is a close association between the use of standard dosages of antimicrobials in livestock and the emergence of resistance to those drugs. Zeina et al. [118] treated cows experimentally with gentamicin and streptomycin and found residues of the antibiotics in a concentration below their MRL in milk after the withdrawal period. All the *Staphylococcus aureus, Escherichia coli*, and *Listeria monocytogenes* they isolated from the milk showed high resistance to gentamicin, and 95% of *S. aureus*, 60% of *E. coli*, and 58% of *L. monocytogenes* isolates were resistant to streptomycin. In milk from non-treated cows, microbial isolates showed, in general, lower levels of resistance. However, they did not perform any statistical analysis to show whether the differences were significant or not. Moreover, a report on seven European states found a strong association between the total use of specific antimicrobial drugs and the level of resistance towards these agents in commensal *E. coli* isolates in pigs, poultry, and cattle [119]. More recently, the third ECDC/EFSA/EMA joint report on the integrated analysis of antimicrobial consumption (AMC) and AR [3] found statistically significant associations between antimicrobial use in animals in EU states and resistant *E. coli* and *Campylobacter jejuni* in the gut microbiota of animals. For *E. coli*, a positive association between AMC and AR was observed in almost all antimicrobial classes. Positive associations between AMC and AR were frequently also found in *C. jejuni*, but not in *Salmonella*.

On the other hand, antimicrobials at low dosages (i.e., residual levels in fed or food, sub-lethal or sub-therapeutic dosages) are also factors contributing to resistance because they promote genetic and phenotypic variability in exposed bacteria [115]. Thus, it has been demonstrated that the meat, meat products, and milk of cows treated with sub-therapeutic concentrations of antibiotics in South Africa had high counts of *Staphylococci* and *Enterobacteriaceae* resistant to streptomycin, methicillin, tetracycline, and gentamicin [120]. Other indirect studies demonstrating the relationship between the exposure to low antibiotic levels and the emergence of resistances are studies with milk, from cows receiving antimicrobial treatment, containing drug residues (waste milk, WM). Tempini et al. [121] found that 20% of *E. coli* isolated from WM showed multiple drug resistance, and only 40% of the isolates were sensitive to all antimicrobials tested. However, no significant association between the presence of drug residues in WM samples and AR in the *E. coli* isolates was found. Other studies, in which pre-weaned calves were fed WM containing very low concentration of antimicrobials, demonstrated that this practice led to increased faecal shedding of antimicrobial-resistant bacteria by calves [122–124].

Focusing on works that study the relationship between the presence of antibiotic residues in milk and dairy products and the appearance of ARB in these foods, the published data are very scarce. For example, in commercial samples, Brown et al. [26] quantified the prevalence of antibiotic residues in pasteurized and unpasteurized milk and ARB in milk sold in Kibera, Kenya. Among unpasteurized milk samples, 23% contained antibiotic residues and 66.7% contained detectable numbers of *E. coli* and, of these, 92.8% were positive for ampicillin and 50% for tetracycline-resistance. However, they did not find any

relationship between the presence of antibiotic residues and the presence of resistances. Zanella et al. [38] also did not find a significant relationship between the presence of antibiotic residues and antibiotic-resistant strains of coliform bacteria in pasteurized cow milk samples in Brazil. Similarly, El Zubeir [125] analysed milk samples for antibiotic residues and ARB in Khartoum, Sudan and found that 20% of samples were contaminated with antibiotics and that isolated bacteria from contaminated milk samples showed a wide range of multiple resistances. However, the authors did not analyse the relationship between the two parameters.

The inconsistency of the results and/or the lack of significant associations is most likely due to the complexity of the problem, as stated before. In all these studies it is impossible to separate AR originating from the pressure exerted by drug residues in the milk from other factors like, for example, those relating to on-farm or processing practices and the environment [120,121].

5. Other Aspects

5.1. Human Health

The presence of antibiotics residues in food, in general, and in milk and dairy products, in particular, may pose a serious threat to human health. The MRL values for antibiotics established by the corresponding authorities are based on the determination of the ADI (acceptable daily intake), which is the amount of a substance that can be ingested daily over a lifetime without appreciable health risk [126] (Figure 1a). Calculation of the toxicological ADI is based on an array of toxicological safety evaluation assays that take into account acute and long-term exposure to the drug and its potential impact on health [14]. Thus, health problems may arise when the MRL is exceeded or drug hypersensitivity reaction occurs.

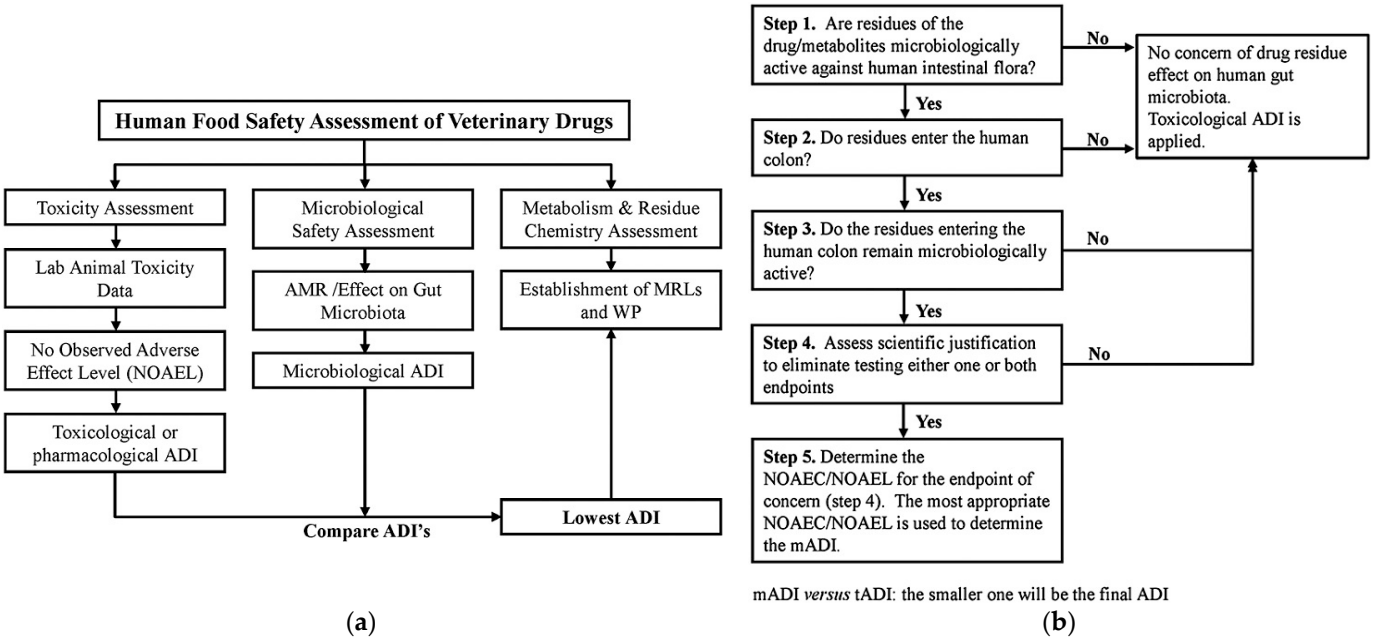

Figure 1. Food safety assessment of veterinary drugs (**a**). Stepwise determination of the need for a mADI (microbial acceptable daily intake) (**b**). ADI: acceptable daily intake; AMR: antimicrobial resistance; mADI: microbial ADI; MRL: maximum residue level; NOAEC: no observed adverse effect concentration; tADI: toxicological ADI; reproduced by permission from John Wiley and Sons [126].

Antibiotic residues may cause various toxic effects like allergy, immunopathological effects, carcinogenicity (sulfamethazine, oxytetracycline, furazolidone), mutagenicity, nephropathy (gentamicin), hepatotoxicity, reproductive disorders, bone marrow toxicity (chloramphenicol), and even anaphylactic shock in humans. All these effects have been

recently reviewed [14,127]. It must be noted that these reviews refer to the health effects of antibiotic residues in food in general. No reviews were found referring to milk or dairy products in particular.

In addition to their direct toxic effects, antibiotic residues can influence both gut microbiota composition and function. Antibiotics at therapeutic doses temporarily alter both the composition of the human gastrointestinal microbiota and the immune and metabolic health of the host [128]. However, the impact of residual concentrations, when ingested either via chronic or acute exposure events, remains very poorly understood [129], and, to the best of our knowledge, no study directed to analyse the impact of residues in specific food, such as milk or dairy products, on human microbiota has been conducted so far.

To establish the MRL values for antibiotics in food, a microbial ADI (mADI) is also estimated (Figure 1b) [126]. The assessment of the mADI for each antibiotic includes the evaluation of two possible effects. One is the capacity of the antimicrobial drug to disrupt the colonization barrier. The colonization barrier is a function of the intestinal microbiome that limits the colonization of the colon by exogenous microorganisms and the overgrowth of indigenous, potentially pathogenic, microorganisms. The second effect is the selection and emergence of AR, that is, the increase in populations of ARB in the gut. This effect may be due either to the acquisition of resistance by organisms which were previously sensitive, or to a relative increase in the proportion of bacteria that are already resistant [126]. Taking into account these two aspects, the no-observed-adverse-effect level (NOAEL) is estimated (Figure 1) in order to establish MRL values for each antibiotic. However, the challenge of this evaluation is to find appropriate methodologies to estimate these effects. To this respect, Piñeiro et al. [129] recently reviewed different aspects of the safety evaluation of veterinary drug residues in animal-derived foods and their effects on the human intestinal microbiome. They also discussed gaps in knowledge and methodology and reviewed the research and scientific approaches being carried out to fill those gaps. For instance, an early study on the subject [130] with murine models showed that the administration of sub-therapeutic antibiotic doses causes changes in the microbiome of young mice and in the copies of key genes involved in the metabolism of carbohydrates to short-chain fatty acids, increases colonic short-chain fatty acid levels, and alters the regulation of hepatic metabolism of lipids and cholesterol, highlighting the risk of feeding milk with antibiotic residues, especially early in life, during the lactation period.

One subject to which special attention has been paid is the role of the food chain in the transfer of ARB and ARG from food to human gut microbiota. As commented before, food contaminated with antibiotics, even at low levels, could be a reservoir of ARB. In addition, such bacteria may be commensal in animals but pathogenic in humans, or may be commensal in both but may later convey resistance to food-borne pathogens in the human gut [131]. However, demonstrating whether ARB in food could pass to the human gut microbiota has been challenging as a consequence of the complex transmission routes of resistances, which include animals, farms, food production facilities, food, and consumers (Figure 2). Nowadays, this study is more affordable thanks to the great development of molecular techniques that allow to detect the same gene in different samples, animals, humans, or food, even if they come from different species [132,133]. In this way, some of the ARG identified in food bacteria have also been detected in the human gut, providing indirect evidence for transfer by food handling and/or consumption. For instance, a study in the Netherlands reported increased levels of ESBL (extended spectrum β-lactamase) enzyme-producing bacteria with similar ARG in poultry meat and humans [134]. No similar work has been found reporting the transference of ARG from dairy product to the human gut microbiota.

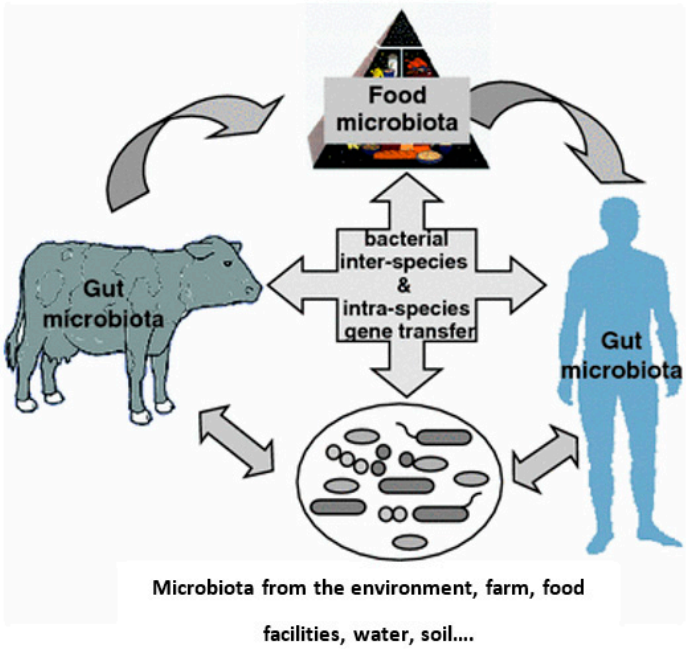

Figure 2. Graphic representation of transmission routes of resistant bacteria and resistance genes, which include animals, farms, food production facilities, food, and consumers (adapted from [112]).

However, several studies and reviews describing the resistance profile of LAB revealed the existence in their genome of mobile elements (plasmids, transposons, and integrons) and insertion sequences, which are responsible for intra- and inter-species transfer of genetic material [135,136]. These kinds of sequences have also been found in cheese [137,138]. In addition, the probiotic potential of many of these bacteria supports the idea of their potential ability to colonize the human gut and transfer ARG within the human gut microbiota, although this fact remains unproven to the best of our knowledge.

5.2. Dairy Farm Environment

Finally, antibiotic residues from dairy animal treatments also have environmental implications, as they can contaminate surface soil when eliminated through whey, urine, and/or faeces, which might affect the local microbiota and groundwater quality, having a big impact in the environment [20]. It has been estimated that 75% to 90% of antibiotics used in food animals are excreted, largely unmetabolized, into the environment and can be detected, for example, in the dust or the ground water of the farms [132]. Besides, by-products of dairies can be recycled. For example, the whey is used in the food manufacturing or animal feeding; manure can be used as fertilizer in vegetable cultivation and transfer residues to crops [139]. Thus, there are many ways by which dairy farming can contribute to the environmental spread of antibiotic residues (Figure 3).

Moreover, ARB present in the intestinal microbiota of farm animals are excreted in manure [140]. Thus, the application of manure in the land or the leaking of waste from storage tanks leads to the spread of ARG in the farm environment. In addition, ARG may be shared between animal, soil, and human bacteria via horizontal gene transfer [141] and, therefore, contribute to the worldwide problem of the increasing AR and multiresistance (Figure 3). However, the contribution of each link in the dairy chain, from farm to fork, to the global problem is still poorly understood [142], and its study is beyond the scope of this review. There are recent reviews and articles dealing with some aspects of this subject [141–143].

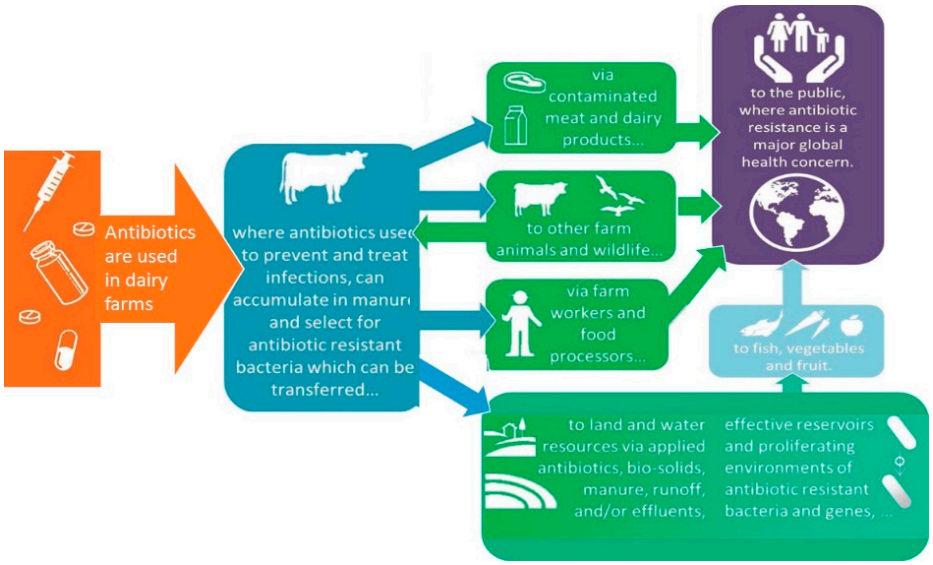

Figure 3. Potential routes of antibiotics and antibiotic resistance transmission, which have their origin in the use of antibiotics in the treatment of farm animals and can reach humans and the environment (adapted from [141] with the permission of Elsevier).

6. Conclusions: So, Where Is the Problem?

The presence of antibiotic residues is a problem with different levels. It is a systemic and widespread problem in developing territories, where regulation is lax or non-existent, constituting a major problem in their food chains. On the other hand, this is an episodic problem in states that have implemented regulation and monitoring systems for these compounds. These programs have reduced the proportion of contaminated food and companies related to its production, reinforcing the idea that its control is possible and desirable. In fact, the education of producers, the awareness of consumers, a guaranteed legal system, an adequate control system, and a "from farm to fork" strategy seem to be the pillars to reduce the concentration of these antibiotic residues throughout the food chain. Even after this strategy has been implemented, the occasional presence of food batches with traces of antibiotics on the market should force governments and institutions to continue supporting programs for their control.

As has been reviewed throughout the manuscript, milk and its derivatives may have antibiotic residues, but their evolution varies among products. The level of antibiotics in yogurts is similar to that present in the milk used for their elaboration. To the contrary, in the case of cheese, most antibiotics go with the whey in the elaboration process. Only aminoglycosides, quinolones, and tetracyclines seem to have a higher susceptibility to be retained in the cheese curd. Nevertheless, the remaining residues in the curd degrade throughout the ripening of cheese. Further research on new techniques/technologies for treating milk that remove antibiotics without affecting quality of dairy products, especially in the case of yogurt, could be the key to addressing this issue. In addition, it would be interesting to study the specific conditions of the production processes to unravel which parameters are important for the degradation or not of antibiotics.

Residues of antibiotics cause delays in the time needed to produce dairy products due to their inhibitory effects on LAB. However, only small changes in the characteristics of the final product have been described.

The development and spread of antibiotic resistant bacteria are the main concerns about the use of antibiotics in dairy animals. Resistant bacteria in the gut of dairy animals and in dairy products can transfer the resistance to pathogenic bacteria in the dairy product and in the human gut microbiota or contribute to its spread in the environment. However, the contribution of each link in the dairy production chain, from farm to fork, to the global problem is still poorly understood. Nevertheless, there is currently active research on the

different aspects of the subject, which gives hope that in the coming years the gaps in knowledge will gradually be filled.

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