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Official controls on carry-over of antibiotics in feed: A useful tool to contain the development of antibiotic resistance

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Abstract

Medicated feeds are widely used to treat bacterial infection in poultry, pig and aquaculture. During feed production process, carry-over can occur as an unintentional but unavoidable presence of traces of active substances in a feed batch to the next ones. Sharing information overall among different countries is a crucial step in order to contain development of microbial drug resistance, which represent a key concern and a research priority. So far, European Union (EU) rules do not provide any carry-over action limit for antibiotics in feed, whereas since 2015 the Italian Competent Authorities established action limits based on the 'as low as reasonably achievable' (ALARA) principles. This study aimed to investigate antibiotics at carry-over level as part of the official feeding-stuffs controls carried out between 2017 and 2020 in North-West of Italy (Piedmont, Liguria and Aosta Valley). Analyses were carried out applying accredited in-house methods, performed with high-performance liquid chromatography with diode-array detector (HPLC-DAD), with fluorescence detector (HPLC-FLD) and with electrospray tandem mass spectrometry (HPLC-MS/MS), to detect different classes of antibiotics. Collected data highlight a decreasing trend of non-compliant samples along the examined period. The most identified compound were tetracyclines which are widely used because of their cost-effective activity. Indeed, swine are confirmed to be the most commonly treated species. Systematic measurement of antimicrobial, at carry-over level in feed is an effective tool to contain the development of antimicrobial resistance. Focused and effective steps on the optimisation antimicrobials use may help to improve feed safety and enhance animal and human welfare.

Introduction

Approved veterinary drugs (i.e., tetracyclines, penicillins, sulfonamides) are widely used in poultry, pig and aquaculture feed to treat bacterial infections [1,2]. Within the EU the use of medicated feeds requires a prescription by authorised professionals, according to the label directions [3]. In Italy veterinarians only are authorized to prescribe antimicrobial treatments through different routes of administration, medicated feed included.

Medicated feeds containing different active ingredients may be processed in the same product plant, so medicated and non-medicated feeding stuffs can be actually produced using a single production line, provided they are cleaned beforehand, in accordance with a documented procedure authorized by the Competent Authority (CA). Nevertheless, traces of active substances or drugs in one batch could eventually persist and transferred in the next feed batches. Carry-over is an unintentional but unavoidable occurrence, which may happen even when identification and control of Hazard Analysis and Critical Control Points (HACCP), and Good Manufactory Practices (GMPs) are followed.

Drug residues in feed, basically antibiotics, may have a deep impact on animal health and on a possible involvement in the development of AMR [4], and it can be expected to occur at each step from primary production, processing, manufacture, storage and feed transport.

Undesirable risks related to this problem are due to possible adverse health effects in target and non-target animals, and therefore in food [2,5]. The use of antimicrobials through animal feed could determine an increased incidence and selection of bacteria resistant to these active compounds [6]. The aftermath of carry-over is an unintentional administration of underdosed antibiotics through feed, which may act as a potential contributing factor to AMR occurrence.

Guidelines for the prudent use of antimicrobials in veterinary medicine [7] claim to avoid systematic use of antibiotics, minimising the development of AMR and limiting their use to those situations where they are indeed required.

In 2019 FAO (FAO Animal Production and Health Report No. 13-2019) [8] pointed out the need of reducing and preventing food safety hazards through a focused monitoring activity, which should be also be carried out on feed.

The scientific risk assessment performed by the European Food Safety Authority (EFSA), the application of GMPs and the ALARA principle have been considered by government regulatory agencies [9] (Reg. EU, 2019/4).

Currently, the EU rules do not provide any carry-over action limit for antibiotics in feed, whereas in Italy, since 2015 and based on the

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ALARA principles, action limits have been established (0.5 mg/kg amoxicillin and ampicillin and 1.0 mg/kg all the other antimicrobials). Moreover, a number of national meetings have been organised in order to improve both the harmonisation of analytical procedures and promoting a consistent approach of feed business operators on this specific issue.

Finally, since 2015 ICA have implemented a National Animal Feed Plan (PNAA) and have enhanced controls on carry-over level detection of additives and antimicrobials in feed. The aim of this study was to provide information on carry-over occurrence in feeding stuffs in our competent territory. Monitoring data collection help to identify factors which may increase the prevalence of the overall antibiotic resistance.

Materials and methods

Data collection and samples selection

Examined data in this study were recorded as part of the official feeding stuffs controls carried out between 2017 and 2020 in the north-west area of Italy (Piedmont, Liguria and). Samples were collected in farms and in feed mills by Local Competent Authorities (LCA), according to Regulation (EC) 691/2013 [10]. In order to gather representative specimens, each lot of feeding-stuffs was repeatedly sampled, taking incremental samples in different single points of the batch and ensuring homogeneity of sampling.

Globally, 785 feed samples were collected and processed: 175 in 2017, 215 in 2018, 229 in 2019 and 166 in 2020. Of these, 61% were complete feed, 18% complementary feed, 15% farm feed and 6% raw materials (i.e. terrestrial animal proteins or fish meal). 59% of collected samples was taken from farm and 41% in feed mills, the most involved species was porcine (42%) (Table 1a and 1b).

Analytical methods

The official samples were analysed for determination of the following antimicrobials: penicillins, quinolones, colistin, amphenicol, lincomycin, macrolides, valnemulin, nicarbazin, sulfonamides, tetracycline, thiamphenicol and tiamulin. Analyses were carried out applying accredited in-house methods, according to UNI CEI EN ISO/IEC 17025. Analytical methods and Limits of Quantification (LOQ) are reported in the Table 2.

Results

Out of the 785 collected samples, 24 were non-compliant to at least one active principle. Of these 75% were complete feed, 16 % farm feed, while 9% were complementary feed; the highest number of non-compliant samples was found in 2017 (n=19) and porcine feed (n=10) appeared to be the most involved category, followed by rabbit (n=5) and bovine feeding-stuff (n=4), while in 2018 (n=3) and 2019 (n=2) all non-compliant samples were porcine feed. Indeed, feed mills irregular samples were higher (n=14) than those found in farm (n= 10) (Table 1b).

As reported in Table 3, tetracyclines appeared to be the more frequently detected antimicrobials during the whole period, while penicillins were found in 2017 only, and other analytes such as nicarbazin, tiamulin, thiamphenicol, sulfadiazine were found just once (Samples ID: 2-CF, 21-CF, 1-CF, 9- CF). Number of non-compliant samples significantly decreased from 2017 to 2019, while no cases were registered in 2020.

In 2019 non-compliant samples were registered for tetracyclines only (samples ID: 23-CF e 24-CF). Lincomycin was detected twice, in 2017 and in 2018 respectively (Samples ID: 3-COMF, 20-CF).

Overall, 54% of the 24 irregular samples showed to contain tetracyclines (10/19 in 2017, 1/3 in 2018, 2/2 in 2019 respectively). Two porcine complete feeds were found to contain both doxycycline and oxytetracycline (14-CF), or doxycycline and chlortetracycline (23-CF).

The amoxicillin concentration in porcine feed in 2017 ranged from 1.3 to 12 mg/kg, while the only ampicillin non-compliant sample was found to contain 7.8 mg/kg (Sample ID 8-CF).

Discussion

This retrospective study highlights that carry-over occurrence of tetracyclines could be rather common in pigs supply chain. However, over the study the percentage of non-compliant samples significantly

Table 1. Sample description (2017-2020): (a) Collected samples, (b) Non-compliant samples

	(a) Collected samples					(b) Non-compliant samples				
	2017	2018	2019	2020	*Total*	2017	2018	2019	2020	*Total*
Sample description										
Complete feed	110	128	136	106	*480 (61%)*	14	3	2	0	*19 (75%)*
Complementary feed	29	30	38	26	*123 (18%)*	1	0	0	0	*1 (9%)*
Farm feed	35	29	23	16	*103 (15%)*	4	0	0	0	*4 (16%)*
Raw materials	1	28	32	18	*79 (6%)*	0	0	0	0	*0*
Total	*175*	*215*	*229*	*166*	*785 (100%)*	*19*	*3*	*2*	*0*	*24(100%)*
Sampling site										
Farm	111	129	131	96	*467 (59%)*	7	2	1	0	*10 (71%)*
Feed mills	65	85	98	70	*318 (41%)*	12	1	1	0	*14 (58%)*
Total	*176*	*214*	*229*	*166*	*785 (100%)*	*19*	*3*	*2*	*0*	*24(100%)*
Involved species										
Porcine	88	92	86	62	*328 (42%)*	10	3	2	0	*15 (62%)*
Poultry	24	36	41	33	*134 (17%)*	0	0	0	0	*0*
Rabbit	25	25	22	17	*89 (11%)*	5	0	0	0	*5 (21%)*
Bovine	23	19	25	17	*84 (11%)*	4	0	0	0	*4 (17%)*
Pets	0	16	28	13	*57 (7%)*	0	0	0	0	*0*
Other species	15	27	27	24	*93 (12%)*	0	0	0	0	*0*
Total	*175*	*215*	*229*	*166*	*785 (100%)*	*19*	*3*	*2*	*0*	*24(100%)*

Table 2. Analytical methods and LOQs

Compounds	LOQs (mg/kg)	Methods
Penicillins	0.50	LC-MS/MS
Tetracycline	0.50	HPLC-DAD
Thiamphenicol	0.50	LC-MS/MS
Lincomycin	0.25	LC/MS
Tiamulin	0.50	LC-MS
Sulfonamides	1.0	HPLC-DAD
Quinolones	0.025	HPLC-FLD
Valnemulin	0.010	LC/MS
Colistin	0.10	LC-MS/MS
Amphenicol	0.10	LC-MS/MS
Macrolides	0.10	LC-MS/MS

Table 3. Non-compliant samples

Year	Sample ID	Species	Classes Of Antibiotics	Compound	Level ± SD (mg/kg)
2017	1-(CF)[1]	PORCINE	Amphenicol	Thiamphenicol	2.4 ± 0.40
	2-(CF)	POULTRY	Coccidiostat	Nicarbazin	14 ± 0.40
	3-(COMF)[2]	BOVINE	Lincosamides	Lincomycin	1.5 ± 0.31
	4-(CF)	PORCINE	Penicillins	Amoxicillin	1.4 ± 0.40
	5-(CF)	PORCINE	Penicillins	Amoxicillin	1.3 ± 0.40
	6-(CF)	PORCINE	Penicillins	Amoxicillin	12 ± 3.3
	7-(FF)[3]	PORCINE	Penicillins	Amoxicillin	2.0 ± 0.60
	8-(CF)	PORCINE	Penicillins	Ampicillin	7.8 ± 2.0
	9-(CF)	BOVINE	Sulfonamides	Sulfadiazine	3.2 ± 0.25
	10-(CF)	PORCINE	Tetracyclines	Chlortetracycline	1.8 ± 0.60
	11-(FF)	PORCINE	Tetracyclines	Chlortetracycline	4.3 ± 0.52
	12-(FF)	RABBIT	Tetracyclines	Doxycycline	6.3 ± 2.0
	13-(FF)	PORCINE	Tetracyclines	Doxycycline	1.3 ± 0.10
	14-(CF)	BOVINE	Tetracyclines	Doxycycline	10 ± 2.0
		BOVINE	Tetracyclines	Oxytetracycline	2.1 ± 0.70
	15-(COMF)	BOVINE	Tetracyclines	Oxytetracycline	11 ± 1.5
	16-(CF)	BOVINE	Tetracyclines	Oxytetracycline	6.3 ± 0.87
	17-(CF)	RABBIT	Tetracyclines	Oxytetracycline	2.6 ± 0.36
	18-(CF)	RABBIT	Tetracyclines	Oxytetracycline	5.6 ± 0.78
	19-(CF)	RABBIT	Tetracyclines	Oxytetracycline	6.2 ± 0.86
2018	20-(CF)	PORCINE	Lincosamides	Lincomycin	5.6 ± 0.91
	21-(CF)	PORCINE	Pleuromutilin	Tiamulin	44 ± 7.5
	22-(CF)	PORCINE	Tetracyclines	Doxycycline	83 ± 6.6
2019	23-(CF)	PORCINE	Tetracyclines	Chlortetracycline	0.61 ± 0.17
		PORCINE	Tetracyclines	Doxycycline	2.8 ± 0.39
	24-(CF)	PORCINE	Tetracyclines	Oxytetracycline	2.8 ± 0.39

[1] CF=complete feed; [2] COMF=complementary feed; [3] FF=farm feed

decreased, and tetracyclines appeared to be the sole antimicrobial class identified in 2019, with no irregular samples detected in 2020.

Many countries have banned antibiotic use as growth promoters, but it has been demonstrated that the use of antibiotics for infection prevention and prophylactic purpose is still rather common in pig production to prevent production loss [11].

In porcine feed penicillins and tetracyclines are the most commonly used antibiotics, due to their cost-effective activity compared to other antibiotics [12]. Their use is often linked to poor plant efficiency and deficient hygiene and welfare conditions, which could result a decreased production and lead to a higher need of antimicrobial treatments [13,14]. The decline of positive samples collected over the years suggests that existing monitoring plans are effective to control carry-over occurrence. Besides, the application of quality management schemes [15,16], more awareness of feed business operators and improved animal welfare conditions appear to contribute positively to a better application of GMP.

Conclusion

The results of our study could be a useful point of view to enhance critical points in feed supply-chain, to ensure a higher level of feed safety and protection of public health and contribute to improve the procedures requirements to minimize carry-over in feed. Our results have been useful shape the ongoing Italian plan (PNAA 2021-2023) for feed monitoring and sampling and might contribute to feed data which could be take into account to improve aware use of antibiotics in farm animals.

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