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Article

Human and Veterinary Antibiotics Used in Portugal—A Ranking for Ecosurveillance

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Abstract: Antibiotics represent a pharmacotherapeutic group widely used in both human and veterinary medicine for which ecosurveillance has been continually recommended. It is urgent to rank the antibiotics and highlight those that may pose potential risk to the environment, a key step for the risk management. The absence of this type of contributions applied to the Portuguese reality supported the idea of compiling the data presented herein. With such purpose the most recent and representative data is used to draw a comparative contribution of each antimicrobial classes according to their intended use, *i.e.*, in human *versus* veterinary medicine. The aim was to assess: (1) the amount and patterns of antimicrobials usage between human and animals; (2) the qualitative comparison between the antimicrobial classes used in each practice (human and veterinary) or specific use; (3)

the potential to enter the environment, metabolism, mode of action and environmental occurrences. This manuscript will, thus, identify priorities for the environmental risk assessment, considering the ranking of the antimicrobials by their usage and potential environmental exposure. Ultimately, this study will serve as a basis for future monitoring programs, guiding the policy of regulatory agencies.

Keywords: antibiotics; consumption; ecosurveillance; environmental occurrence; human medicine; risk ranking; pharmacokinetic features; Portugal; veterinary medicine

1. Introduction

Antibiotics are bioactive compounds, of both a natural and semi-synthetic nature, belonging to the antimicrobial group. Although in the European Union (EU) their use is restricted to the treatment of diseases in humans and animals (given their antibacterial activity), they are further used, in other parts of the world as growth promoters and to improve feed efficiency in livestock.

The golden era of antibiotics' discovery was the period between 1940s and 1970s. Since then, their usage has continuously risen in both human and veterinary medicine. Indeed currently antibiotics are the pharmacoterapeutic group more widely administered in both human and veterinary medicine. Nevertheless, the decline of the discovery rate associated with the growing emergence of antibiotic resistant microorganisms is of concern. In human medicine it resulted in high mortality rates observed in the multidrug-resistant bacterial infections in humans and the ensuing extra health care costs. In veterinary medicine it may compromise the viability of intensive livestock production because of the potential for the rapid spread of disease and the use of antibiotics as a metaphilaxic tool. Currently, there are more than 10 different groups of antibiotics which are characterized according to their structural and chemical properties. Their mechanisms of action are also varied, for instance interference with the cell membrane, cell wall synthesis, folic acid metabolism, protein synthesis, and DNA-dependent RNA polymerase or DNA replication of bacteria [1–3]. It has been recognized that the prolonged use of some classes of antibiotics in clinically relevant doses (e.g., quinolones, aminoglycosides) can induce effects on oxidative damage of DNA, proteins synthesis and membrane lipids of mammals [4]. However, exposure to antibiotics it is not exclusive of antibiotic consumption through prescribed individual treatments as recent investigations highlight the detection of antibiotics in the environment which is explained by their high excretion (up to 90%) in the unchanged form via urine and/or feces. The antibiotic environmental contamination can contribute further to the increased emergence of resistance in pathogenic and environmental bacteria [5,6] and toxic effects in humans. Extrapolating the expected effects resulting from the prolonged use in mammals, chronic effects on aquatic animals could not be excluded.

The antibiotics are released to the environment through different pathways and the environmental exposure route for humans and veterinary medicinal products are distinct. Once metabolized mainly by liver, part of the antibiotic administered dose is excreted in feces and urine as its original active substances and/or metabolites [7]. Furthermore, some of the excreted metabolites can be transformed back into the original or other active substance [8]. Whereas the antibiotics administered in humans are

mainly conducted to sewage treatment plants (STPs) and, if not removed, reach the environment by their effluents, the antibiotics used in animal production may be excreted directly into the environment, or accumulate in manure which could later be spread on land as fertilizer [9,10]. Thus, the environmental exposure to veterinary antibiotics seems to result in a "hot-spot" format, with concentrations higher than the environmental exposure to human antibiotics. Nevertheless, hospitals also contribute extensively to the environmental contamination. High prevalence of bacteria resistant to beta-lactams (e.g., cefotaxime and ceftazidime) has been detected in hospital effluents. Many antibiotics end up in surface water or in sediments where they can leach out into the surrounding water depending of their photostability, binding, adsorption capacity and degradation. Data collected in environmental water samples revealed concentrations of antibiotics ranging from ng/L up to mg/L depending on the sampling site and weather conditions [11,12].

Although antibiotic contamination of surface water has been found, it is not yet known if the low chronic concentrations investigated are able to cause imbalance/damage on the ecological system. Some recent studies demonstrated that antibiotics present in sewage cause a negative impact in bacteria population associated with biological processes within STPs [13]. Because of the potential health effects brought about by possible exposure to these contaminants, there is a growing interest in implementing survey programs to monitoring pharmaceuticals in the environment [14,15].

In the late 1990s, it was performed the first quantitative analysis of both human and veterinary antimicrobials usage to compare the data and indirectly estimate the antimicrobial resistance. In the following years a similar approach has been performed by other EU countries [16].

Furthermore, some studies have demonstrated that the presence of pharmaceuticals in the environment correlates well with their used amount in both human and veterinary medicine. Therefore, this data can be used to identify antibiotics that may pose a risk to the environment [17,18].

This manuscript provides, for the first time, an assessment of the antibiotic usage, through the most recent Portuguese official sales data, in both human and veterinary medicine. It is also provided a brief review of the most important pharmacological and environmental features of each antibiotic, along with their environmental occurrence reported in Portugal and Europe. With such information, antibiotics will be ranked and the active substances that pose the possible risk to the environment identified. Ultimately, this manuscript aims to conduct an antibiotic risk-based ranking in Portugal, as an important tool for the ecosurveillance.

2. Materials and Methods

2.1. Antimicrobial Consumption

In the scope of the present manuscript, and considering that in Portugal the antibiotics are sold as prescription-only medicines, it was assumed that antimicrobial consumption was paralleled by the officially reported sales, as retrieved from the national human and veterinary medicine's regulators, specifically the Portuguese National Authority of Medicines and Health Product (INFARMED) and the National Authority for Animal Health (DGAV). The period in study corresponded to the consecutive years of 2010 and 2011.

For Veterinary Medicine, data was collected from the national reports of monitoring of antimicrobial consumption [19,20]. As laid down in Portuguese national regulation on veterinary medicines, a report on the veterinary use of antimicrobials has to be available on an annual basis. Such report gathers data on the basis of a questionnaire sent to every authorized wholesaler that distributed veterinary medicinal products containing antimicrobials for the period between January and December of the year in question. The provided data on the consulted reports was presented in the context of the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC project) as included in the reflection paper on collecting data from EMA [21].

As to human medicine, for the same period under study, the Department for the Medicine's Economic Assessment of the INFARMED provided information on antibiotics sales data by package, pharmaceutical form and quantitative composition, thus allowing estimation of the amount of active substance for each antibiotic therapeutic group [22]. The collected data refers exclusively to the medicines of systemic use in the primary health care sector, *i.e.*, medicines dispensed in ambulatory, within the Portuguese National Health Service. All pharmaceutical forms and administration routes were included, except antibiotics for topic use which were not covered by the present study. Regrettably, the nonexistence of unitary available data regarding antibiotics of exclusive use in the hospital sector prevented estimation of the corresponding antibiotic amount consumed. Nevertheless, and given its significance, their amounts were systematized in a percentage distribution and included in the estimated antimicrobial ranking.

To simplify the comparison between the amounts of active substances used for both human and veterinary practice, the unit of measurement chosen was tonnage.

2.2. Key Pharmacokinetic Features and Occurrence of the Most Representative Antibiotics

To perform the ranking a focused review of the major pharmacological features that can determine the extent of the environmental impact of the most consumed antimicrobials in the period in question was carried out. Equally for the leading active substances consumed in Portugal, recent European reports on the environmental occurrence were summed up.

2.3. Antibiotic Ranking Approach

The ranking approach for the antibiotics described in the present paper, had as first rational criteria for their selection the outcome of the predicted environmental risk assessment in surface water, *i.e.*, crude predicted environmental exposure (PEC) in surface water. It results from the consumption data for every active substance and the number of Portuguese inhabitants at the year 2010 and 2011 (results not shown). The following equation was used [23]:

$$[\text{crude PEC } (\mu g/L) = [A \times (F_{\text{excreta}})]/(Q_{\text{effluent}} \times \text{hab} \times \text{Dilution} \times 365] \quad (1)$$

where A is the amount of human antibiotic used per year (2010 and 2011); F_{excreta}, the excretion fraction of the antibiotic (100%); Q_{effluent}, the amount of wastewater per inhabitant per day (default value: 200 L inhab/day); *hab*, the number of inhabitants in Portugal for 2010 and 2011; Dilution, the dilution from STPs effluents to surface water (default value: 10); 365, the days per year.

Thus, only the antibiotics used in human medicine which had a crude PEC greater than the trigger value of 0.01 μg/L, meaning with potential surface water environmental risk, were selected. In this exercise, it has been observed that the minimal annual amount for which the corresponding PEC is slightly above the action limit of 0.01 μg/L was 0.090 tonnes. Therefore, this threshold was employed for the selection of veterinary active substances herein.

Every selected antibiotic was correlated with their parent compound percentage excretion and the occurrence. The total 59 substances gathered were categorized in three lists, as follows:

Rank (1) Antibiotics used only in human medicine
Rank (2) Antibiotics used only in veterinary medicine
Rank (3) Antibiotics used in both sectors (human and veterinary medicine).

3. Results

3.1. Antimicrobial Consumption

The estimated antibiotic amounts for human primary health care sector and veterinary use considering their route of administration and target species in the case of veterinary medicines are displayed on Table 1.

The annual amount of antibiotics used in human medicine in primary health care sector was 81.4 tonnes in 2010 and 85.9 tonnes in the following year (2011). The consumption remained at the same level for both years (merely 3.0% of difference) and with roughly the same pattern analyzing the distribution per classes of antibiotics. Only the quinolones group increased in the year 2011 (from 8.6 to 13.4 tonnes), being the consumption of ciprofloxacin the major determinant.

Considering the two years under study together (2010 + 2011), the annual used amount of the different therapeutic groups was markedly larger for penicillins, which alone accounted for more than 65.0%, followed by quinolones (13.0%), macrolides (7.0%), cephalosporins (6.0%) and sulfonamides (5.0%).

The most representative active substances by therapeutic group were: cefaclor, cefatrizine, cefuroxime and cefadrine (cephalosporins); azythromycin, clarithromycin (macrolides); amoxicillin, flucloxacillin (penicillins); ciprofloxacin, norfloxacin, prulifloxacin (quinolones); sulfamethoxazole, trimethoprim (sulfonamides and trimethoprim) and fosfomycin (other antibiotics).

In veterinary medicine the annual amount of antibiotics sold was 179 tonnes in 2010 and 163 tonnes in 2011. This 9% decrease was mainly attributed to a lower amount of oxytetracycline (tetracycline group), which compared with the previous year, accounted for less than 33.2 tonnes. Nevertheless, tetracyclines (doxycycline, oxytetracycline) together with penicillins (amoxicillin, ampicillin, benzylpenicillin) were the most used therapeutic groups for the both years. The consumption of lincosamides (clindamycin) increased significantly, from 1.7 tonnes (2010) to 16.7 tonnes (2011). Likewise, macrolides, pleuromutilins and quinolones raised 61.0%, 45.0% and 41.0% for 2011, respectively, comparing with the year of 2010.

Regarding the amount for the remaining active substances, a significant decrease of their use compared with the previous year was mainly observed for colistin and sulfadiazine, which decreased 48.0% and 58.0%, respectively.

Table 1. Estimation of antibiotic consumption in the human primary health care sector and in veterinary practice by route of administration and target species in the period of 2010–2011.

Active substance	Human Medicine			Veterinary Medicine			Target species [25]	Amount [2010 + 2011] (tonnes)
	Amount (tonnes)		Administration route [24]	Amount (tonnes)		Administration route		
	[2010]	[2011]		[2010]	[2011]	[25]		
Aminoglycosides								
Apramycin	---	---	---	1.020	0.761	Oral use (drinking water)	Porcine; bovine; poultry;	**1.781**
Dihydrostreptomycin (Streptomycin) $^{+H}$	---	---	---	0.526	0.425	Injection use	Porcine; bovine; ovine; caprine; equidae; rabbit; canine; feline;	**0.951**
Gentamicin^{+H}	0.004	0.000	Injection use	0.068	0.035	Injection use	Porcine; bovine;	**0.107**
Neomycin $^{+H}$	0.001	0.000	Oral use (tablet); Injection use	0.274	1.903	In-feed use [a)]	Porcine; poultry;	**2.178**
Netilmycin $^{+H}$	0.004	0.001	Injection use	---	---	---	---	**0.005**
Paromomycin $^{+H}$	0.000	0.000	---	0.00	0.326	Intramammary use	Bovine	**0.326**
Spectinomycin	---	---	---	0.019	0.028	Injection use Oral use (drinking water)	Porcine; bovine; ovine; caprine; equidae; canine; feline; Porcine; poultry;	**0.047**
Total amount	**0.009**	**0.001**		**1.021**	**3.478**			
Amphenicols								
Florfenicol	---	---	---	1.491	1.487	Injection use In-feed use	Porcine; Bovine Porcine	**2.978**
Cloramphenicol $^{+H}$	0.000	0.000		0.000	0.000			**0.00**
Total amount				**1.491**	**1.487**			
Antituberculosis								
Etambutol	0.003	0.004	Oral use (tablet)	---	---	---	---	**0.007**
Pirazynamide	0.001	0.002	Oral use (tablet)	---	---	---	---	**0.003**
Rifampicin	0.041	0.043	Oral use (capsule); lyophilisate for infusion	---	---	---	---	**0.084**
Total amount	**0.045**	**0.049**						

Table 1. *Cont.*

Active substance	Human Medicine			Veterinary Medicine			Target species [25]	Amount [2010 + 2011] (tonnes)
	Amount (tonnes)		Administration route [24]	Amount (tonnes)		Administration route [25]		
	[2010]	[2011]		[2010]	[2011]			
Cephalosporins								
Cefaclor $^{+H}$	0.780	0.550	Oral use (capsule, Granules)	---	---	---	---	**1.330**
Cefadroxil $^{+H}$	0.463	0.374	Oral use (capsule)	0.003	0.004	Injection use	Bovine; equidae; canine;	**0.844**
Cefalexin	---	---	---	0.481	0.220	Injection use Intramammary use	Bovine; feline; canine; Bovine	**0.701**
Cefalonium	---	---	---	0.009	0.011	Intramammary use	Bovine	**0.020**
Cephapirin	---	---	---	0.005	0.003	Intramammary use	Bovine	**0.008**
Cefatrizine $^{+H}$	0.766	0.622	Oral use (capsule; tablet; liquid suspension)	---	---	---	---	**1.388**
Cefazolin $^{+H}$	0.00	0.00	---	0.004	0.003	Intramammary use	Bovine	**0.007**
Cefditoren	0.052	0.033	Oral use (tablet)	---	---	---	---	**0.085**
Cefeprozil	0.344	0.086	Oral use (tablet)	---	---	---	---	**0.430**
Cefixime $^{+H}$	0.452	0.408	Oral use (tablet; liquid suspension)	---	---	---	---	**0.860**
Cefonicide	0.004	0.004	Injection use					**0.008**
Cefoperazone	---	---	---	0.012	0.010	Intramammary use	Bovine	**0.022**
Cefquinome	---	---	---	0.099	0.064	Injection use Intramammary use	Porcine; bovine; equidae; Bovine;	**0.163**
Cefodizime	0.004	0.004	Injection use	---	---	---	---	**0.008**
Cefotaxime	0.005	0.00	Injection use	---	---	---	---	**0.005**
Cefovecin	---	---	---	0.002	0.002	Injection use	Canine; feline;	**0.004**
Cefradine $^{+H}$	0.927	0.890	Oral use (capsule)	---	---	---	---	**1.817**
Cefuroxime $^{+H}$	1.477	1.473	Oral use (tablet); Injection use	---	---	---	---	**2.950**
Ceftiofur	---	---	---	0.198	0.252	Injection use	Porcine; bovine;	**0.450**
Ceftriaxone $^{+H}$	0.000	0.123	Injection use	---	---	---	---	**0.123**
Total amount	**5.278**	**4.571**		**0.813**	**0.569**			

Table 1. *Cont.*

Active substance	Human Medicine			Veterinary Medicine			Target species [25]	Amount [2010+2011] (tonnes)
	Amount (tonnes)		Administration route [24]	Amount (tonnes)		Administration route [25]		
	[2010]	[2011]		[2010]	[2011]			
Macrolides								
Azithromycin $^{+H}$	1.375	1.466	Oral use (tablet)	---	---	---	---	**2.841**
Clarithromycin $^{+H}$	3.365	3.274	Oral use (tablet)	---	---	---	---	**6.639**
Erythromycin $^{+H}$	0.462	0.414	Oral use (tablet)	0.013	0.017	Injection use	Bovine	**0.906**
Gamithromycin	---	---	---	0.020	0.021	Injection use	Bovine	**0.041**
Roxithromycin	0.042	0.050	Oral use (tablet)	---	---	---	---	**0.092**
Spiramycin	0.648	0.563	Oral use (capsule) Injection use	0.278	0.279	Injection use	Porcine; bovine; ovine; caprine	**1.768**
Telithromycin	0.018	-----	Oral use (tablet)	---	---	---	---	**0.018**
Tildipirosin	---	---	---	0.000	0.001	Injection use	Porcine; bovine;	**0.001**
Tilmicosin	---	---	---	3.288	5.071	Injection use Oral use (drinking water)	Bovine Porcine; bovine; poultry	**8.359**
Tylosin	---	---	---	9.906	16.302	Injection use Oral use (drinking water) In-feed use	Porcine; bovine; ovine; caprine; Porcine; bovine; poultry; Porcine; bovine; poultry;	**26.208**
Tulathromycin	---	---	---	0.014	0.054	Injection use	Porcine; bovine	**0.068**
Total amount	**5.910**	**5.767**		**13.519**	**21.745**			
Lincosamides								
Clindamycin	0.175	0.159	Oral use(capsule) Injection use	0.001	0.001	Capsule	Canine	**0.336**
Lincomycin	0.004	0.005	Oral use (capsule)	1.713	16.731	Injection use Oral use (drinking water) In-feed use	Porcine; bovine; ovine; caprine; equidae; canine; feline Porcine; Poultry Porcine	**18.453**
Total amount	**0.179**	**0.164**		**1.714**	**16.732**			
Monobactams								
Aztreonam	0.015	0.008	Injection use	---	---	---	---	**0.023**
Total amount	**0.015**	**0.008**						

Table 1. *Cont.*

Active substance	Human Medicine			Veterinary Medicine			Target species [25]	Amount [2010+2011] (tonnes)
	Amount (tonnes)		Administration route [24]	Amount (tonnes)		Administration route [25]		
	[2010]	[2011]		[2010]	[2011]			
Penicilins								
Amoxicillin [+H]	49.603	50.901	Oral use (tablet, liquid suspension)	24.868	21.227	Injection use Oral use (drinking water) Oral use (tablet)	Porcine; bovine; canine; feline; Porcine Canine	**146.599**
Ampicillin [+H]	0.186	0.276	Injection use	4.557	2.894	Injection use Oral use (drinking water) [d)]	Porcine; bovine; ovine; equidae; canine; feline Bovine; ovine	**7.913**
Benzylpenicillin [+H]	0.131	0.131	Injection use [b,C)]	4.909	2,184	Injection use [e)] Intramammary use [f)]	Porcine; bovine; ovine; equidae; canine; feline; Bovine	**7.355**
Cloxacillin [+H]	---	---	---	0.359	0.071	Intramammary use [g)]	Bovine; ovine; caprine;	**0.430**
Dicloxacillin [+H]	0.012	0.008	Oral use (tablet)	---		---	---	**0.020**
Flucloxacillin [+H]	4.130	3.596	Oral use (capsule)	---	---	---	---	**7.726**
Phenoxymethylpenicillin [+H]	0.000	0.000	---	0.144	0.021	Oral use (drinking water)	Poultry	**0.165**
Pivmecillinam	0.013	0.008	Oral use (tablet)	---	---	---	---	**0.021**
Total amount	**54.075**	**54.92**		**34.837**	**26.397**			
Polymyxins								
Colistin [+H]	0.000	0.000	---	15.408	8.013	Injection use Oral use (drinking water) In-feed use	Porcine; bovine; canine Porcine; bovine; ovine; poultry Porcine; poultry; rabbit	**23.421**
Total amount				**15.408**	**8.013**			
Polypeptides								
Bacitracin [+H]	0.000	0.000	---	0.000	1.710	In-feed use	Porcine; bovine; poultry; rabbit	**1.710**
Total amount					**1.710**			

Table 1. *Cont.*

Active substance	Human Medicine			Veterinary Medicine			Target species [25]	Amount [2010 + 2011] (tonnes)
	Amount (tonnes)		Administration route [24]	Amount (tonnes)		Administration route [25]		
	[2010]	[2011]		[2010]	[2011]			
Quinolones								
Ciprofloxacin	5.963	10.940	Oral use (tablet)	---	---	---	---	**16.903**
Danofloxacin	---	---	---	0.043	0.049	Injection use	Bovine	**0.092**
Difloxacin	---	---	---	0.001	0.000	Oral use (tablet)	Canine	**0.001**
Enrofloxacin	---	---	---	5.644	8.386	Injection use Oral use (tablet) Oral use (drinking water)	Porcine; bovine; canine; feline; Canine; feline Porcine; poultry; rabbit	**14.03**
Flumequine	---	---	---	0.675	0.470	Oral use (drinking water)	Bovine; poultry	**1.145**
Levofloxacin [+H]	0.804	0.832	Oral use (tablet)	---	---	---	---	**1.636**
Lomefloxacin	0.005	0.004	Oral use (tablet)	---	---	---	---	**0.009**
Marbofloxacin	---	---	---	0.040	0.117	Injection use	Porcine; bovine; canine; feline	**0.157**
Moxifloxacin [+H]	0.224	0.202	Oral use (tablet)	---	---	---	---	**0.426**
Norfloxacin [+H]	0.748	0.678	Oral use (tablet)	---	---	---	---	**1.426**
Ofloxacin [+H]	0.140	0.116	Oral use (tablet)	---	---	---	---	**0.256**
Oxolinic acid	---	---	---	0.003	0.000	Oral use (pellet)	Fish	**0.003**
Pradofloxacin	---	---	---	0.000	0.001	Oral use (tablet, liquid suspension)	Canine; feline	**0.001**
Prulifloxacin	0.726	0.626	Oral use (tablet)	---	---	---	---	**1.352**
Total amount	**8.61**	**13.398**		**6.406**	**9.023**			
Sulfonamides and Trimethoprim								
Sulfadiazine [+H]	0.005	0.008	Oral use (tablet)	10.478	4.428	Injection use Oral use (drinking water) In-feed use Oral use (paste)	Porcine; bovine; ovine; equidae Porcine; poultry Porcine Equidae	**14.919**
Sulfaguanidine	---	---	---	0.017	0.026	---	---	**0.043**
Sulfadoxine	---	---	---	0.030	0.036	Injection use	Porcine; bovine; ovine; equidae; canine; feline	**0.066**
Sulfaquinoxaline	---	---	---	0.022	0.028	Oral use (drinking water)	Rabbits	**0.050**
Sulfamethoxazole [+H]	3.971	3.764	Oral use (tablet)	---	---	---	---	**7.735**
Trimethoprim [+H]	0.794	0.753	Oral use (tablet) [h)]	1.545	3.040	Injection use [i)]	Porcine; bovine; ovine; equidae; canine; feline	**6.131**
Total amount	**4.770**	**4.523**		**12.092**	**7.558**			

Table 1. *Cont.*

Active substance	Human Medicine			Veterinary Medicine			Target species [25]	Amount [2010+2011] (tonnes)
	Amount (tonnes)		Administration route [24]	Amount (tonnes)		Administration route [25]		
	[2010]	[2011]		[2010]	[2011]			
Tetracyclines								
Doxycycline [+H]	0.242	0.247	Oral use (capsule)	28.115	28.273	Oral use (drinking water) In-feed use Oral use (tablet)	Porcine; bovine; poultry Porcine Canine; feline	**56.877**
Minocycline	0.260	0.256	Oral use (tablet)					**0.516**
Oxitetracycline	---	---	---	46.539	13.329	Injection use Oral use (drinking water) Intrauterine use Cutaneous use (spray) Nebulisation use	Porcine; bovine; ovine; equidae; canine; feline; Porcine; bovine; ovine; caprine; Porcine; bovine; ovine; caprine; equidae Porcine; bovine; ovine; caprine; equidae Porcine; bovine; ovine; caprine; equidae; poultry; rabit; canine; feline	**59.868**
Tetracycline [+H]	0.000	0.000	---	2.001	2.860	Oral use (drinking water) [j]	Porcine; bovine; ovine; caprine; poultry	**4.861**
Total amount	**0.502**	**0.503**		**76.655**	**44.462**			
Pleuromutilins								
Tiamulin	---	---	---	14.659	16.302	Injection use Oral use (drinking water) In-feed use	Porcine Porcine; poultry; Porcine; rabbit;	**30.961**
Valnemulin	---	---	---	0.113	5.075	In-feed use	Porcine	**5.188**
Total amount				**14.772**	**21.377**			
Other antibiotic								
Fosfomycin	1.613	1.748	Oral use	---	---	---	---	**3.361**
Fusidic acid	0.303	0.279	Oral use (granule)	---	---	---	---	**0.582**
Rifaximin	---	---	---	0.012	0.011	Cutaneous use (spray) Intramammary use	Bovine Bovine	**0.023**
Total amount	**1.916**	**2.027**		**0.012**	**0.011**			

[+H], also hospital use; [a], It could be associated to streptomycin or bacitracin; [b] It could be associated to clavulanic acid; [c] associated to phenoxymethylpenicillin; [d] It could be associated to colistin; [e] It could be associated to streptomycin; [f] It could be associated to framycetin; [g] It could be associated to cloxacillin and colistin; [h] Associated to sulfamethoxazole ; [i] It could be associated to sulfadiazine or sulfadoxine; [j] It could be associated to sulfametazine. Benzylpenicillin = Penicillin G ¸phenoxymethylpenicillin = Penicillin V.

Figure 1. Comparison between antibiotics consumption in both human (orange) and veterinary (green) medicine in the two years under study and the total consumption taking into account both practices (purple) (TMP, trimethoprim).

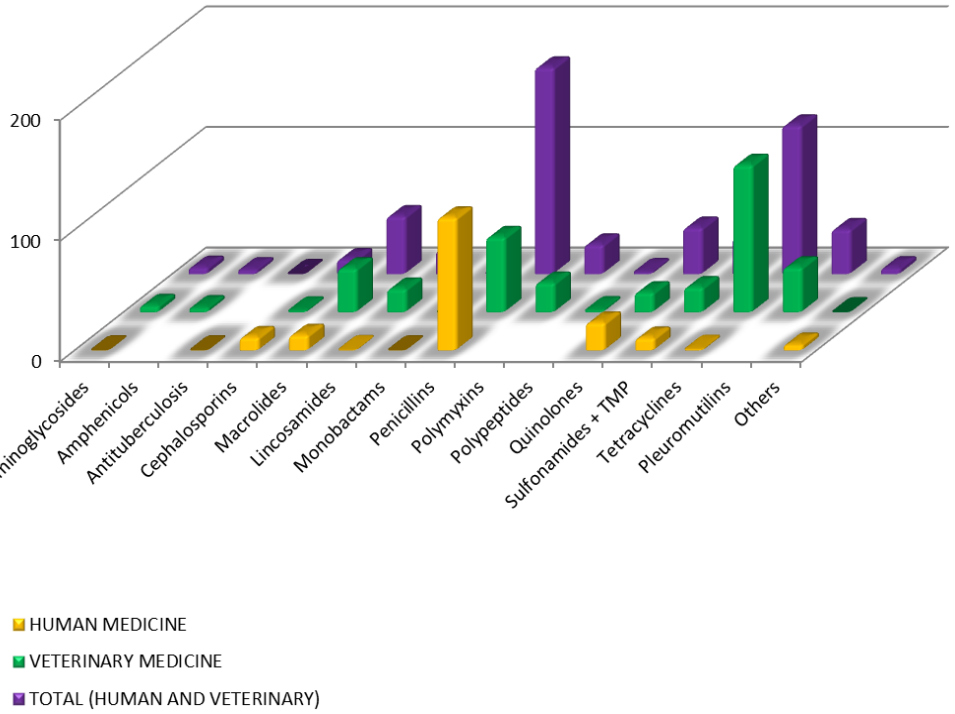
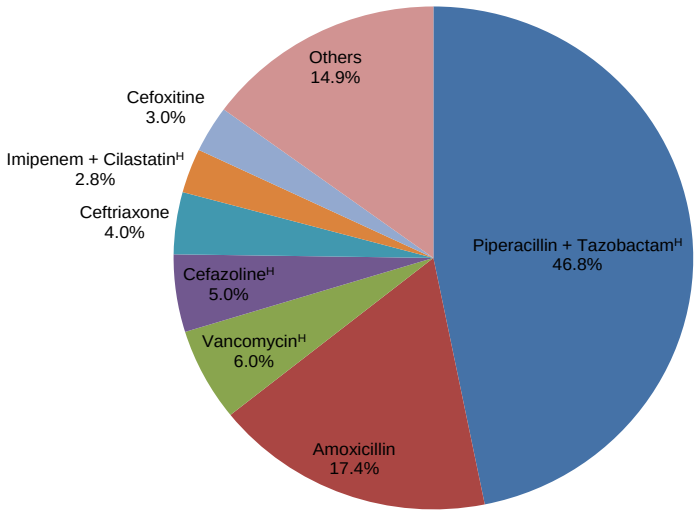


Figure 2. Distribution of the most used antibiotics within the hospital care sector in the studied years of 2010 and 2011 (^{H}, Hospital exclusive use).



Comparing the amount of antibiotics sold in each practice (Figure 1) it is evident that the more consumed antibiotics in human medicine belonged to the penicillin class and in veterinary medicine to the tetracycline class.

In both years 2010 and 2011 the total consumption of antimicrobials used in hospitals was estimated in ~22.0 tonnes. However, this value could be surpassed if missing data from one major central hospital had been provided. Seven active substances dominated the hospital antimicrobial use

(Figure 2): piperacillin in combination with tazobactam (46.8%), amoxicillin (17.4%), vancomycin (6.0%), cefazoline (5.0%), ceftriaxone (4%), cefoxitine (3.0%) and imipenem in combination with cilastatin (2.8%).

Antibiotics of exclusive hospital use, for instance amikacin, colistin methanesulphonate sodium, sulbactam, linezolid, teicoplanin, tetracycline, tigecycline, tinidazole or tobramycin, showed individual negligible percentages in the collected data.

3.2. Key Pharmacokinetic Features and Occurrence of the Most Representative Antibiotics

Besides the amount consumed, the pharmacokinetic features of an antimicrobial, particularly its bioavailability (% F) and proportion of excretion as parent compound, contribute to a major or lesser environmental impact and are related with the corresponding reported occurrence in different environmental compartments. For that reason, the main pharmacokinetic features were summed up in Table 2.

Table 3 presents a review of the occurrence of antimicrobials, across Europe according to recently reported in different environmental compartments such as influents and effluents of wastewater treatment plants (WWTP), surface water, groundwater, drinking water, sediments/sludge, soils and animal manure.

3.3. Antibiotic Ranking Approach

Table 4 summarizes the antibiotic ranking results. Comparing the three Ranks (human, animal, and total), it could be observed that the majority of antibiotics used in both medicines correlate positively with the occurrence in the environment. Furthermore, the percentage of excretion in unchanged form is relatively high for many of these substances. Some of these antibiotics were detected in high concentrations mainly in hospital waste water. From the antibiotics included in Rank 1, only 8 out of 24 have been reported in environmental studies (Tables 3 and 4). Vancomycin was the only antibiotic from hospital exclusive use found in occurrence studies.

Similarly, environmental occurrence of the antibiotics used in veterinary medicine (Rank 2 of Table 4) is mainly reported in waste water compartments (Table 3), whereas studies of occurrence in sediment, soil, manure are lacking in most cases.

Furthermore, in the estimated ranking it is readily noticeable that the top-ranked antibiotics are precisely those used in both human and veterinary medicine (Rank 3 of Table 4). Of the 14 listed antibiotics, 10 (71%) where found in the environment.

Common features between the top-ranked antibiotics listed in Rank 1 (Ciprofloxacin) and Rank 3 (amoxicillin) are the frequent environmental detection and high percentage of excretion as the unchanged form.

Shortly, from the ranking performed in this paper for the selected antibiotics it could be concluded that (1) the antibiotics authorized for human and veterinary medicine are those with higher potential environmental impact; (2) there is lacking studies of occurrence for a considerable number of antibiotics authorized in Portugal.

Table 2. Brief characterization usage, pharmacokinetic (PK) properties and environmental considerations of selected antibiotics.

	Key Features
AMINOGLYCOSIDES	**Characterization**: Aminoglycosides consumption in human medicine is mostly reserved to hospital care (e.g., gentamicin, streptomycin, neomycin, paromomycin, tobramycin). In veterinary medicine aminoglycosides are mainly used in drinking water (e.g., apramycin) and in feed (e.g., neomycin) for the treatment of bacterial gastrointestinal infections manly in calves, pigs and poultry. **PK**: Due to their highly polar and cationic nature aminoglycosides are poorly absorbed from intestinal tract and undergo negligible biotransformation when administered parenterally. Its pharmacokinetic profile is similar for humans and animal species. After oral administration the absorption of neomycin in calves was minimal (1%–11%) and about 90% was recovered in faeces (70%–80% as the parent compound). The urinary recovery of paromomycin (aminosidine) ranges from 80%–100% of total administered dose [26,27]. **Environment**: Fate and behavior studies in environment as well as monitoring results on their occurrence in surface or wastewater were not found. However, aminoglycosides are of special interest because of their potential impact on the spread and maintenance of antimicrobial resistance. It has been demonstrated that bacteria (enterococci) isolates from environmental samples (groundwater, wastewater) were resistant against gentamicin and streptomycin [28].
AMPHENICOLS	**Characterization**: The most used active substance for this group is florfenicol, which is administered to farm animals by intramuscular (IM) route or in feed, mainly in cattle and porcine. **PK**: Cattle have similar kinetic profile as pigs. Florfenicol is absorbed completely and rapidly though the gastrointestinal (GI) tract in pigs, however oral bioavailability reported in broiler chickens is lower (55%–71%). After administration in farm animals four metabolites were identified (florfenicol-amine, florfenicol alcohol, florfenicol oxamid acid and monocloride florfenicol) but the only relevant microbiologically active residue is florfenicol. The main elimination route of florfenicol is urine, with 45%–60% of a dose excreted as parent drug, 11%–17% as florfenicol-amine, <10% was eliminated as florfenicol oxamid acid and 1% as florfenicol alcohol [29–32]. **Environment**: Fate and behavior studies in environment as well as monitoring results on their occurrence in soil, surface or wastewater are scarce. However, considering an open environmental assessment for the veterinary medicinal product Nuflor™, florfenicol showed an average biotransformation in soil of 13 days and in water greater than 28 days (d). The degradation by hydrolysis or photolysis (direct or indirect) was not significant and the degree of soil sorption was also considered low [33]. According to Subbiah *et al.* [34] residual florfenicol in soil can exert selective effect on bacteria (*E. coli*). In occurrence studies florfenicol was not detected.

Table 2. *Cont.*

	Key Features
CEPHALOSPORINS	**Characterization**: Cephalosporines are mainly used in Portugal in human medicine (Table 1) available mostly as tablets, granules or capsules to treat urinary tract infections due their high concentration in urine. In veterinary species they are frequently used for intramammary treatment of clinical mastitis in lactating cattle (Table 1), although ceftiofur is also available for administration by intramuscular route in porcine and bovine. **PK**: The pharmacokinetic profile of cephalosporins may vary between molecules and between species. The major route of excretion is renal filtration with the exception of cephalothin, cephapirin, and cefotaxime (which are deacetylated) and ceftriaxone and cefoperazone which are eliminated by bile. In some instances, about 90% of active substance is excreted unchanged [35,36]. **Environment**: Cephalosporines are subject to hydrolytic cleavage of the β-lactam ring under alkaline conditions being less sensitive to hydrolysis than penicillins. Similar to penicillins and tetracyclines, they can be complexed with cations and accumulate in sewage sludge and sediments [37], what may explain the bacterial resistance (*E. coli*) against cephalosporins observed in sewage treatment plants (e.g. cefuroxime) [38]. Cephalosporins are degraded with half-lives <5 d in the water sediment. However the degradation rate in the sediment was substance specific, for instance, cefradine was degraded in 0.87 t½ (d) and cefuroxime in 2.6 t½ (d). Abiotic processes seem to play an important role in the elimination of cephalosporins in surface water. The degradation of cephalosporins in environment is higher at higher pH values [39]. Hydrolysis of ceftiofur in soils was accelerated by increasing pH; the *t*½ values at pH 5.7 and 9 were ±100, 8 and 4 days respectively, at 22 ℃ [40,41].
MACROLIDES	**Characterization**: In human medicine the most predominant macrolides were azithromycin, clarithromycin. Tylosin and tilmicosin are solely used in veterinary medicine mainly in drinking water and incorporated in feed. Spiramycin and erythromycin are used for both human and animal practice. **PK**: Macrolides are quite lipophilic molecules that undergo extensive hepatic metabolism. Metabolism of erithromycin is via hepatic microsomal enzymes and only 2%–5% of orally administered erythromycin is excreted in active form in the urine. Clarithromycin is eliminated by renal and non-renal mechanisms and metabolized to several metabolites. In urine the amount of clarithromycin excreted unchanged ranges from 20%–40%, and 10%–15% of the dose is excreted as 14-hydroxyclarithromycin. Azithromycin undergoes some hepatic metabolism to inactive metabolites, but biliary excretion is the major route of elimination [42–44]. Oral bioavailability of tylosin is low with 22.5% in pigs and 30%–34% in broiler chickens. Tylosin is metabolized by liver and excreted mainly via faeces with only 6% of the parent compound being excreted by urine. Several compounds (tylosin A, tylosin Factor B, C and D and metabolite) may quantitatively differ between formulations and species. Tilmicosin has slow and low absorption (22%). After administration of a single oral dose of tilmicosin to pigs about 80% is excreted in faeces and 15% in urine. Similarly, in sheep, 72% are excreted in faeces and 13% in urine [45–47]. **Environment**: Their extensive administration in farm animals, could lead to contamination of manure and the subsequent contamination of soils whenever used as fertilizer. Schuesener *et al.* [48] studied the persistence of erythromycin, tylosin and roxithromycin in soil. Degradation half-lives of 20 days and 8 days were determined for erythromycin and tylosin respectively, however the concentration of roxithromycin remained unchanged after 120 days. Considering this study erythromycin and tylosin are not persistent in soil. Also in manure tylosin and tilmicosin were not detected being rapidly degraded. Both active substances are weak bases that are unstable under alkaline and acid conditions but are relatively stable at neutral conditions. The same review concluded that tylosin sorption in sediment also increased by the raising in ionic strength and is very immobile in sandy loam and loam sediments [48].Therefore, their hydrolysis depends of the physical-chemical properties of manure and sludge. It has been reported that tylosin and erythromycin does not modify the ribosomal mechanism responsible for resistance of soil environmental bacteria [48–50]. In environment, erythromycin is transformed in dehydro-erythromycin. Azithromycin, clarithromycin and roxithromycin are degraded via direct photolysis. Azithromycin under photolysis turns into other photo products which exhibit a lower antibiotic activity than the parent compound [51,52]. Loads of spiramycin, dehydro-erythromycin and roxithromycin in treated wastewater were generally lower than in untreated water, because they can bind to sludge and will be released later [53].

Table 2. *Cont.*

	Key Features
LINCOSAMIDES	**Characterization:** Lincosamides are basic compounds with high lipid solubility which include lincomycin and clindamycin. Both substances are used in human and veterinary medicine. Lincomycin is administered mainly in hospital sector and in drinking water or in feed for animals destined to human consumption. In humans lincomycin is used orally but has currently been largely replaced by clindamycin. **PK**: Lincosamides are well absorbed from intestines of non-herbivores and are eliminated mainly by hepatic metabolism (e.g., S-oxidation, de-methylation) although about 20% is excreted as unchanged form by urine. Lincomycin oral absorption in swine was in the range of 20%–50%, similarly to humans. The main component of the urine in human is the unchanged lincomycin (4%–17%). In pigs, about 11%–21% was excreted into the urine: 50% as unchanged lincomycin, and trace amounts of *N*-demethyl lincomycin. Chickens treated orally showed that excreta contained 80% lincomycin, ≤10% lincomycin sulfoxide, ≤5% *N*-demethyl lincomycin [42,54,55]. Clindamycin metabolites are much like those described for lincomycin. The bile is the major excretion route of clindamycin. About 28% excreted by the liver in the glucuronide form, 28% as clindamycin sulfoxide, and 9% as *N*-demethyl clindamycin [42]. **Environment**: The pK_a of these substances is about 7.6, indicating that they will exist partially in the cation form which could absorb more strongly to soils containing organic carbon and clay than their neutral counterparts. No behavior studies in environment could be found for these substances. Studies demonstrated that clindamycin degraded in the sediment with a half-life of 1.0–1.6 days [56,57].
PENICILLINS	**Characterization:** Penicillins are widely used in human health care and hospital sector such as in veterinary medicine in target animals. Amoxicillin is the most frequently administered active substance from this class (~147 tonnes 2010 and 2011). **PK**: Amoxicillin is usually administered by oral route in humans and animals due to its good absorption from the GI tract. This good absorption is attributed to its free amino group and zwitterionic ionisation at physiological pH, as amoxicillin exhibit low lipophilicity. Large interspecies differences in oral bioavailability of amoxicillin have been reported ranging from 10% in horses to 25%–30% in pigs and calves, while 63% in chickens and 75% to 80% in humans. Renal excretion (tubular secretion) is the primary elimination route as for most β-lactam antibiotics and their metabolites. Penicillin G, Penicillin V, ticarciline and aminopenicillins are metabolized to some extent by hydrolysis of the β-lactam ring. The two major metabolites found are amoxicilloic acid (AMA) and amoxicillindiketopiperazine-2',5'-dione (DIKETO)[58–61]. **Environment**: Penicillins have a similar environmental behavior as cephalosporins. They are highly polar substances and consequently would not be expected to sorb readily to soil components. Due to the chemically unstable β-lactam ring they are susceptible to hydrolysis and therefore easily degraded [62]. However, they have been occasionally detected in the environment.
PLEUROMUTILINS	**Characterization**: The most used active substances for this group are tiamulin and valnemulin, both consumed solely in veterinary medicine. **PK**: Tiamulin undergoes extensive metabolism in the liver and no antimicrobial activity was observed for 67% of the metabolites. Metabolite 8-α-hydroxymutilin was identified as residue marker. In dogs 33% of tiamulin dose was excreted in the urine [63–65]. Valnemulin is also excreted rapidly, mostly via the bile and faeces (around 87% of the total dose). In pigs valnemulin is extensively metabolized and excretion of parent molecule and metabolites occurs mainly via bile (73%–95% of the daily dose of total was recovered from the faeces). In rabbits, valnemulin is extensively metabolized with the same metabolites being found as in pigs [66–68]. **Environment**: Schuesener *et al*., studied the persistence of tiamulin in soil. Degradation half-lives of 16 days were determined. Considering this study tiamulin could not be seen as persistent in soil. However, in manure tiamulin was detected for about 180 days, showing no degradation. Thus, the soil will be contaminated with tiamulin if the manure is used as fertilizer [48].

Table 2. *Cont.*

	Key Features
POLYMYXINS	**Characterization**: The polymyxins are a group of *N*-monoacetylated decapeptides that includes polymyxin B and polymyxin E (colistin). These peptides are considered as basic surface-active cationic detergents with an ability to intercalate in phospholipid which results in cell death (bactericidal properties). Despite being available for clinical use since the 1950s, the increasing prevalence of multidrug resistance worldwide has led to the resurgence of its use. **PK**: Polymyxins are not absorbed from the GI tract. Therefore systemic therapy in humans requires that a parenteral preparation (either polymyxin B sulfate or colistin methanesulphonate) be injected. Polymyxins are slowly excreted unchanged by glomerular filtration in urine. On the basis of the findings in earlier studies, approximately 60% of the dose of polymyxin B was recovered in urine [42,55,69]. Although it has been used regularly in veterinary medicine for decades for the treatment of GI infections, its use is now being questioned given the ever growing need for antimicrobials for the treatment of multidrug-resistant infection in humans. Colistin sulphate is poorly absorbed after oral administration and following parenteral administration excretion is via urine with no detectable residues found in faeces [70,71]. **Environment**: One study demonstrated the resistance of *Staphylococcus aureus* isolates from medical hospital, veterinary hospital, and slaughterhouse waste effluents against colistin [72].
QUINOLONES	**Characterization**: The most predominant active substances are: ciprofloxacin, levofloxacin, moxifloxacin, norfloxacin, ofloxacin and prulifloxacin solely aplicated in human medicine and enrofloxacin and flumequine administered in food animals, mainly in drinking water. Interestingly ciprofloxacin although not authorized for animal use is an important active metabolite of enrofloxacin. **PK**: The majority of fluoroquinolones are primarily excreted in urine as the parent compound, by glomerular filtration or tubular secretion. However urinary excretion of these quinolones may be highly variable presenting 7% to 90% as fraction of unchanged drug excreted in urine. Ciprofloxacin and levofloxacin, the most representative fluoroquinolones for human use, are mainly eliminated by kidneys presenting 33%–60% and 61%–83% of urinary excretion, respectively. Only 15% of a ciprofloxacin dose has been reported to be recovered in feces. In contrast, moxifloxacin exhibit very little renal elimination. Only 20% of a moxifloxacin dose was recovered unchanged in the urine. After an oral dose 25% of a moxifloxacin dose was recovered as unchanged drug in feces partially. Furthermore, prulifloxacin is a prodrug of the active metabolite ulifloxacin. The proportion of ofloxacin dose excreted in urine is about 70% [73–76]. Enrofloxacin is metabolized to ciprofloxacin via deethylation of the ethyl group on the piperazine ring. About 13%–60% of enrofloxacin is metabolized into ciprofloxacin (20%–50%) and smaller amounts (around 10%) of oxygenated or dealkylated ciprofloxacin and hydrolylated enrofloxacin. Although in pigs there were only small traces, in cattle, the proportion of ciprofloxacin metabolized from enrofloxacin in plasma has been measured as high as 25%. Therefore around 90% of the applied enrofloxacin is excreted into environment in the active form either as parent compound or its active metabolite ciprofloxacin [42,77]. In addition, oral bioavailability in broiler chickens is 57% for norfloxacin, 64% for enrofloxacin and 69% for ciprofloxacin [60]. **Environment**: Except for prulifloxacin, quinolones exhibit large chemical stability, do not degrade with increased temperature and do not undertake hydrolysis. Fluoroquinolones, despite the low octanol-water coefficient values, bind strongly to sludge, probably through metal complexes interactions, influencing their solubility. Ofloxacin, compared to other fluoroquinolones, adsorbs strongly to different types of soils and is not easily released. A recent study demonstrated that their degradation could be enhanced under nitrifying conditions. Prulifloxacin showed extensive degradation in hydrolytic and oxidative processes, being stable to thermal and photolytic stress conditions [78–80].

Table 2. *Cont.*

	Key Features
SULPHONAMIDES + TRIMETHOPRIM	**Characterization**: The sulfonamides have been applied in composite drugs with trimethoprim (TMP). **PK**: The treated humans and animals excrete the sulphonamides as parent compound or as acetylated derivatives. All sulfonamides (except the enteric compounds oral use) are excreted by kidneys. Orally used sulfonamides are primarily excreted via feces, with little or no drug being absorbed systemically. Acetylation is the major pathway of sulfonamides in most species, and apparently acetylated metabolites are the major urinary metabolites in cattle, sheep and swine. Glucuronide conjugation and aromatic hydroxylation are two additional metabolic pathways by which sulfonamides are metabolized in animals. In calves and cows, sulfadiazine is acetylated to a great degree with lower concentration of 4-hydroxysulfadiazine and with no glucuronides or 5-hydroxy derivatives detected in this species [81–86]. In pigs, two main metabolites were reported *N*-acetylsulfadiazine and 4-hydroxysulfadiazine and two minor metabolites identified as *N*-formylsulfadiazine and *N*-acetyl-4-hydroxysulfadiazine. Sulfadiazine accounted for 44% of the 96% excreted [87]. TMP is rapidly absorbed after oral administration, being the oral bioavailability reported as 79% in chickens and 73% in pig [83–85]. The greater part of the dose is excreted as unchanged drug and around 10%–20% of a dose is metabolized and metabolites are mainly excreted in the urine as conjugates. The fraction of TMP dose excreted as unchanged compound in humans is 69% ± 17 % while in veterinary use reported values varies from 2% in goat, 3% in cow, 10% in horses, 16% in pigs and 20% in dogs [55,88]. **Environment**: Sulphonamides are polar and hydrophilic, being transferred into the aquatic environment. They are considered the most mobile of antibiotics. They exhibit weak sorption to soil and so does not easily adsorb onto activated carbon. In environment the most predominant sulphonamide was sulfamethoxazole . In the waste water could be found the parent compound and its metabolite N^4-acetylsulfamethoxazole, which it is assumed to be retransformed to the antibiotic itself by abiotic processes. After sulphametazine acetylation, the acetylated compound undergoes conjugation to form the glucuronide of *N*-4-acetylated sulfamethazine. Sulfamethazine metabolite, glucuronide of *N*-4-acetylated sulfamethazine is converted back to the parent drug in liquid manure. The abiotic degradation (indirect photolysis) found to be the main elimination pathway. They are classified as photo- and thermally stable substances at the degradation half-life (DT50) > 1 year [81,89–91]. TMP was also found to be susceptible to indirect photolysis in wastewater effluents. However is highly stable to direct photolysis and very stable photo-transformation products are generated during this process [92].

Table 2. *Cont.*

	Key Features
TETRACICLYNES	**Characterization**: Doxycycline has gained importance due of its higher bioavailability relative to other tetracyclines. In veterinary medicine this broad-spectrum antibacterials are mainly used in drinking water and in food for animals. **PK**: Biovailability vary between species and molecules. In pigs, oxytetracycline, tetracycline and chlortetracycline biovailability is very low: 3%, 18%, 11% pigs, respectively [93]. In broiler chickens and pigs oral bioavailability of doxycycline was 41%, 48%, respectively, which was lower than that found in humans (90%–95%) [60,94]. The primary route of elimination for most tetracyclines is the kidney (with doxycycline being an important exception) although they are also excreted by bile into intestines, where they are partially reabsorbed via enterohepatic recirculation. Minoclycline is an exception and is significantly metabolized in the liver with low renal clearance. The tetracyclines (except minocycline and doxycycline) are excreted unchanged in the urine by glomerular filtration. About 10%–35% of a dose of oxytetracycline is excreted in active form in the urine and 20%–60% of a dose of tetracycline was reported. In most domestic animals biotransformation of the tetracyclines seems to be limited, and generally about one-third of a given dose is excreted unchanged. Sheep excrete nearly 21% of an oral dose of oxytetracycline, and young bulls excrete about 17%–75% of chlortetracycline as parent compound. Doxycycline and minocycline may be more extensively biotransformed than other tetracyclines (up to 40% of a given dose). Generally 50%–80% of a given dose is recoverable from the urine. Intestinal (biliary) elimination is always significant, it is commonly ~10%–20%, even with parenteral administration; for doxycycline and its metabolites, and minoclycline, this is the major route of excretion [43,95,96]. **Environment**: The tetracyclines just like penicillins, readily precipitate as complex forms with cations, as calcium and magnesium and were accumulated in sludge or sediment. Studies have shown that tetracyclines can absorb strongly to different kind of soil (loam, clay), sediment, being considered immobile, like enrofloxacin, ciprofloxacin and ofloxacin [97]. Szatmári *et al*., found degradation half-lives of 66.5 days for doxycycline in soil fertilized with liquid manure. Smaller values have been reported for oxytetracicline and for tetracycline: about 17 days and 22 days respectively. This difference indicates a higher adsorption to the soil particles of doxycycline. Temperature, UV and pH might contribute to the degradation of tetracyclines [98,99].
BACITRACIN	**Characterization**: Bacitracin zinc is an antibiotic from the group of peptides widely used in veterinary medicine mainly incorporate in feed of farm animals (1.7 tonnes for 2012). **PK**: After oral application bacitracin is hardly absorbed from gastrointestinal tract and approximately 95% of an oral dose is excreted via faeces (only 3% or less via urine). It is metabolized to amino acids and smaller peptides via the main metabolite desamidobacitracin (inactive). Main metabolites in faeces are bacitracin A, B1, B2, F, desamidobacitracin and catabolic peptides [100]. **Environment**: Given its low stability constant, bacitracin zinc would be expected to dissociate into zinc ions and free bacitracin under environmental conditions [81].

Table 2. *Cont.*

	Key Features
OTHER: HOSPITAL USE	**Characterization**: Imipenem, linezolide, piperacillin and vancomycin are miscellaneous antibacterial mostly used in hospital settings for serious infections. Imipenem is a carbapenem antibiotic with a broad spectrum of activity particularly useful for critically ill patients with septic complications due to unidentified bacteria or bacteria resistant to many other antibiotics. Linezolid belongs to a new class of antibacterial agents, the oxazolidinones, which are active against a variety of gram-positive pathogenic bacteria, including methicillin-resistant strains of *Staphylococcus aureus*. Piperacillin is an extended-spectrum penicillin which combines a ureidopenicillin and a beta-lactamase inhibitor. Vancomycin primarily administered via the intravenous route in the critical care setting in human medicine. In this setting, vancomycin has been mainly used to treat infections where MRSA is the presumed cause of infection. **PK**: Imipenem is rapidly metabolized by the renal brush border enzyme dehydropeptidase I. It is therefore co-administered as a one-to-one combination with the enzyme inhibitor cilastatin, which results in the excretion of about 70% of unchanged imipenem into the urine [101]. Linezolid and the two main metabolites have been reported to be predominantly eliminated by the kidneys. Its urinary recovery (% of dose) after 24 hours was much as 51.3% [102,103]. Both piperacillin and tazobactam undergo hepatic metabolism; two metabolites formed by beta-lactam ring cleavage contribute little (10%) to the overall elimination of piperacillin and about 26% of an metabolite of tazobactam was found in healthy volunteers. The recovery of piperacillin and tazobactam in urine over 12 h was 42.5% and 60.0% of the corresponding administered doses, respectively. Biliary route may also play important role piperacillin elimination [104]. Due to its poor absorption after oral administration vancomycin may also be used in some GI conditions (e.g., *Clostridium difficile* diarrhea). With normal renal function, about 90% of intravenous vancomycin is eliminated unchanged in the urine during the first 24 h by glomerular filtration. By intravenous route no vancomycin was observed in faeces [105]. **Environment**: Data about the fate and degradation in environment was not found. Vancomycin was the only one detected in wastewater and surface water (see Table 3).

Table 3. Environmental concentrations levels (ng/L or μg/kg) of antibiotics most commonly used in Europe and Portugal according to recent literature. Only the highest values reported for each environmental compartment in each country were included.

Active substance	Marketing authorization in Portugal		Environmental concentration levels per sphere (ng/L or μg/kg)								European country [Reference]
	Hum. Med.	Vet. Med.	Wastewater influent	Wastewater effluent	Surface water	Groundwater	Drinking water	Sediment/sludge	Soil	Manure/Dung	
AMPHENICOLS											
Florfenicol		X		n.d.							EU [106]
CEPHALOSPORINS											
Cephalexin		X		283	n.d.	n.d.					Serbia [106]
MACROLIDES											
Azithromycin	X		0.33 295	101.0 1.04 [HS] 7351 [HS]	1620						France [26] Italy [107] Portugal [108] Spain [109]
Clarithromycin	X		319.0 52.3	374 117.0 960 [HS]	20.3 0.26 20.5 18.2	n.d.	n.d.	0.16			Italy [27,110,111] Germany [112] France [26] Portugal [108] Spain [109] Serbia [113]
Erythromycin	X	X	12.0 220	52.0 0.03 [HS] 7545 [HS] n.d.	15.9 1.70 111.9 292	n.d.	n.d.	0.18			Italy [27,110] Germany [29] Spain [114,115] Portugal [108] Serbia [113]
Roxithromycin	X		0.14	13 [HS]	0.56 23.8						Germany [29] Italy [107,111] Spain [109]
Spiramycin	X	X	603.0	454.0	74.20 2980			0.66			Italy [27,28] Spain [109]
Tilmicosin		X	0.46	0.35 [HS] 93.2	2.5 820			n.d.			Italy [27,107] EU [106] Spain [109]
Tylosin		X	n.d.	n.d. [HS] n.d.	2.77 n.d.			n.d.			Italy [27,28,107] EU [106] Spain [109]
LINCOSAMIDES											
Lincomycin	X	X	9.7	6.1 317	248.9			n.d.			Italy [27,28] EU [106]
Clindamycine	X	X		277							EU [106]

Table 3. *Cont.*

Active substance	Marketing authorization in Portugal		Environmental concentration levels per sphere (ng/L or µg/kg)								European country [Reference]
	Hum. Med.	Vet. Med.	Wastewater influent	Wastewater effluent	Surface water	Groundwater	Drinking water	Sediment/sludge	Soil	Manure/Dung	
PENICILLINS											
Amoxicillin	X	X	18.0	n.d. 40.0 n.d.	5.7			n.d.			Italy [27] France [26] EU [106]
Ampicillin	X	X	252	 n.d.							Portugal [116] EU [106]
Benzylpenicillin	X		127	 n.d.							Portugal [116] EU [106]
Phenoxymethylpenicillin	X *			122							EU [106]
Cloxacillin		X		n.d.							Germany [30]
Dicloxacillin	X			n.d.							Germany [30]
QUINOLONES											
Ciprofloxacin	X		 513.0 330 300.0	 499 38689 [HS] 101 [HS] 101.0 124.5 [HS] 264 434 278	443 8.8 119.2 28.2	 n.d.	 n.d.	7.3 3 2.0	4.6		Spain [31,109] Italy [27,111] Portugal [108,117,118] Sweden [108,119] France [26,28] Germany [120] EU [106] Switzerland [121,122] Serbia [113]
Enrofloxacin		X	447.1 n.d.	211.5 [HS] n.d. [HS] n.d.	102.5 264				 0.37	 8.3	Portugal [28,117,118] Italy [107] EU [106] Spain [109] Austria [123]
Norfloxacin	X		 174.0 0.31	37 0.51 [HS] 388	n.d. 462			 6.8	 8.4		Portugal [28] Sweden [28] Italy [107] Spain [31,109] Switzerland [122]

Table 3. *Cont.*

Active substance	Marketing authorization in Portugal		Environmental concentration levels per sphere (ng/L or µg/kg)								European country [Reference]
	Hum. Med.	Vet. Med.	Wastewater influent	Wastewater effluent	Surface water	Groundwater	Drinking water	Sediment/sludge	Soil	Manure/Dung	
Ofloxacin	X				1903			2.7	3.3		Spain [31,115]
			463.0	235.0	10.9			3.4			Italy [27]
			4986	24810 [HS]							Portugal [108]
			287.0	7.6 [HS]		n.d.					Sweden [28,119,124]
				510.0	3.2						France [26,124]
				220	n.d.	n.d.	n.d.				Serbia [113]
Danofloxacin		X	n.d.	n.d. [HS]							Italy [107]
					543						Spain [109]
Flumequine		X		25.7							EU [106]
					10.3						Spain [109]
SULFONAMIDES AND TRIMETHOPRIM											
Sulfadiazine	X	X			4.33						Germany [29]
			0.026	0.38 [HS]	236						Italy [107,125]
										91	Austria [123]
				105							EU [106]
				208							Spain [109]
Sulfamethoxazole			11.60	0.40	0.48	410					Germany [29,30,120]
	X				119.3			1.1	n.d.		Spain [31,115]
			246	185	402.0		80.0	n.d.			Italy [27,28,111]
			155	39.0	5.0						Luxemburg [28]
			674	304							Sweden [28]
				205.0	1.9	3.0					France [26,124]
			1662	8714 [HS]	53.3						Portugal [108,126]
				432	n.d.	n.d.	n.d.				Serbia [113]
Sulfadoxine		X		n.d.							EU [106]
Trimethoprim	X	X			0.32						Germany [30]
				30 [HS]	252			1.6	0.2		Spain [31,114,115]
			0.072	72	0.9	1.4					France [26,107,124]
										n.d.	Switzerland [127]
										17	Austria [123]
			80	40							Sweden [128]
			360	3963 [HS]	15.7						Portugal [108,126]
				39							Italy [111]
				800							EU [106]
				259	8.1	n.d.	n.d.				Serbia [113]

Table 3. *Cont.*

Active substance	Marketing authorization in Portugal		Environmental concentration levels per sphere (ng/L or µg/kg)								European country [Reference]
	Hum. Med.	Vet. Med.	Wastewater influent	Wastewater effluent	Surface water	Groundwater	Drinking water	Sediment/sludge	Soil	Manure/Dung	
TETRACYCLINES											
Oxitetracycline		X	n.d.	n.d. 1.3 [HS] n.d.	4.49 41 1.1			n.d. n.d.	n.d. n.d. n.d.	136 29	Germany [30,129] UK [29] Spain [31,109] Italy [27,107] Austria [123] EU [106]
Doxycycline	X	X	n.d.	6.7 [HS] 0.97 [HS] n.d.	188						Sweden [119] Italy [107] EU [106] Spain [109]
Tetracycline		X	n.d. 32.3	n.d. n.d. n.d. [HS] 22.8 [HS]	141			6.5	n.d. n.d.	23	Germany [30] Spain [31,109] Austria [123] Italy [107] Portugal [108]
OTHERS											
Vancomycin [H]	X		41	40	4.8				n.d.		Italy [107]

n.d. not detectable; [HS] Hospital samples; * in combination with benzylpenicillin; [H] Exclusive hospital use.

Table 4. Ranking of the most consumed antibiotics (above 0.090 tonnes), correlated with their parent compound excretion (%) and environmental occurrence (Occ.), used only in human medicine (Rank 1; including of exclusive hospital use, marked [H]) and veterinary medicine only (Rank 2) and active substances authorized for both practices (Rank 3), in the two studied years.

Rank 1 **Human antibiotic**	**Amount (tonnes)**	**% Exc. Unchanged form**	**Occ.**	**Rank 2** **Veterinary antibiotic**	**Amount (tonnes)**	**% Exc. Unchanged form**	**Occ.**	**Rank 3** **Human + Veterinary antibiotics**	**Amount (tonnes)**	**% Exc. Unchanged form**	**Occ.**
Ciprofloxacin	16.90	60	+	Oxytetracycline	59.87	35	+	Amoxicillin	146.60	90	+
Piperacillin [H]	~9.20	43	n.f.	Tiamulin	30.96	33	n.f.	Doxycycline	58.88	70	+
Flucloxacillin	7.73	-	n.f.	Tylosin	26.21	6	+	Lincomycin	18.45	49	+
Sulfamethoxazole	7.73	15	+	Colistin	23.42	>90	n.f.	Sulfadiazine	14.92	44	+
Clarithromycin	6.64	40	+	Enrofloxacin	14.03	90	+	Ampicillin	7.91	60	+
Fosfomycin	3.36	-	n.f.	Tilmicosin	8.39	15	+	Benzylpenicillin	7.36	90	+
Cefuroxime	2.95	40	n.f.	Valnemulin	5.19	-	n.f.	Trimethoprim	6.13	69	+
Azithromycin	2.84	12	+	Tetracycline	4.86	60	+	Neomycin	2.18	80	n.f.
Cefradine	1.82	-	n.f.	Florfenicol	2.98	60	n.d.	Spiramycin	1.77	20	+
Levofloxacin	1.64	83	n.f.	Apramycin	1.78	>90	n.f.	Erythromycin	0.906	10	+
Norfloxacin	1.43	30	+	Bacitracin	1.71	<10	n.f.	Cefadroxil	0.844	10	n.f.
Cefatrizine	1.39	-	n.f.	Flumequine	1.15	55	+	Clindamycin	0.336	10	+
Prulifloxacin	1.35	-	n.f.	Dihydrostreptomicin	0.951	-	+	Paromomycin	0.326	>90	n.f.
Cefaclor	1.33	80	n.f.	Cephalexin	0.701	87	+	Phenoximethylpenicillin	0.165	-	n.f.
Vancomycin [H]	~1.28	90	+	Fusidic acid	0.582	-	n.f.				
Tazobactam [H]	~1.08	60	n.f.	Minocycline	0.516	60	n.f.				
Cefixime	0.86	-	n.f.	Ceftiofur	0.450	80	n.f.				
Imipenem/Cilastatin [H]	~0.73	70	n.f.	Cloxacillin	0.430	-	n.d.				
Minocycline	0.516	60	n.f.	Cefquinome	0.163	83	n.f.				
Cefeprozil	0.430	-	n.f.	Marbofloxacin	0.157	-	+				
Moxifloxacin	0.426	20	n.f.	Danofloxacin	0.092	-	+				
Ofloxacin	0.256	-	+								
Linezolide [H]	~0.154	51	n.f.								
Roxythromycin	0.092	66	+								

([H] *Hospital exclusive use;* + found; n.d. not detectable; n.f. not found in our literature review; % Exc. Unchanged form refers to the proportion of the molecule excreted through urine as parent compound, considering the maximum values reported in Table 2).

4. Discussion

In our study, we performed a "first-step" ranking for the most consumed antibiotics in human and veterinary medicine in Portugal. As emphasized previously, it is of paramount importance to identify the antibiotics for which data on environmental occurrence and the ensuing possible risk is missing.

Given the scope of the present paper, data on antibiotic consumption was converted in amounts (tonnage) to compare the both human and veterinary practice. Due to the employed methodological approach, comparison of consumed antibiotics with previously reported data is hindered, given that the latter is always provided as defined daily dose (DDD; a WHO statistical measure of drug consumption). Additionally, analysis of hospital amounts was limited by the lack of some data. Nonetheless, estimated trends give important insights, by highlighting the active substances that may be significant for future research in environmental surveillance approaches.

Analyzing and comparing antibiotic usage in human and veterinary medicine in Portugal it is readily noticeable that about two-thirds of the consumed antibiotics are used in veterinary medicine whereas only one-third in human medicine. The veterinary usage trends have been kept with moderate variation during the last years. Indeed, the amount of antibiotics sold in veterinary practice in the two years under study (179 tonnes—2010 and 163 tonnes—2011) is of the same order of magnitude of the ones previously reported for 2006 (166 tonnes, [130]).

Mainly for veterinary medicine, obvious variations between the 2010 and 2011 have been observed on usage trend of some pharmacotherapeutic groups, which could be related to animal demography, epidemiological situations, and use of new molecules over old substances (e.g., lincosamides, macrolides).

Some antibiotic classes used in both practices may be limited to specific use in target animal species but with a significant usage in humans (e.g., cephalosporins). However, due to the emergence of multi-drug resistance of bacteria, the human antibiotics are being increasingly subjected to restricted prescription towards an almost exclusive hospital use. Therefore, the hospitals waste waters could currently be considered as a "hot-spot" for these substances. In the current study, fifty nine antibiotics were selected for the ranking approach, as described on section 2.3. Thus, the minimal amount chosen for starting the ranking has been 0.090 tonnes, which corresponds to a crude PEC surface water value for the human antibiotics slightly above the action limit of 0.01 μg/L [131]. In the case of veterinary antibiotics the crude PEC calculation concerns mostly the soil [132] instead of the surface water. However, we extrapolated the calculated minimal amount of 0.090 tonnes for the veterinary antibiotics, because it seems conservative enough to cover the main purpose of the present study.

Every top ranked substances in Rank 1 (referring to human and hospital use antibiotics) and Rank 3 (antibiotics used in both practices) correlates positively with occurrence reports. In Rank 3 the two mentioned top-ranked active substances (amoxicillin and doxycycline) are characterized by pharmacokinetic features that may concur to their occurrence in the environment as they both present high proportion of parent compound excreted (>70%). Amoxicillin belongs to the penicillin group which undergoes little metabolic reactions, being excreted primarily by kidney by tubular secretion. Doxycycline is also characterized by high percentage of excretion in the unchanged form, although intestinal excretion may also be relevant (Table 2). On the other hand, the top of Rank 1 is occupied by ciprofloxacin. This quinolone is also highly excreted by urine as parent compound being also

considered to have high proportion (60%) of unchanged form in the urine. Some studies report even higher values up to 83.7 [133].

Conversely, oxytetracycline, the top-ranked antibiotic of Rank 2 (antibiotics used in veterinary practice only) features a moderately low (35%) percentage of the parent compound available in urine (Table 2). Considering this percentage of excretion in unchanged form, an environmental exposure was not likely. However, the fact was that the amount of oxytetracycline consumed in the two studied years was 60 tonnes in veterinary practice. Accordingly, oxytetracycline was the tetracycline with the highest levels reported in animal manure as found in Germany (136 μg/kg) and Austria (29 μg/kg) (Table 3). Indeed oxytetracycline is used by oral route in drinking water to treat porcine, bovine, ovine and caprine species. In addition, its corresponding oral bioavailability values reported in bibliography were very low in pigs (3%) and sheep (21%) as stated before [93–96]. This could mean that a great extent of dosage form could be excreted directly to excreta from those animals due to their low fraction absorbed to blood. The same consideration can be applied to tylosin (also listed on Rank 2) which is mainly metabolized by liver and excreted mainly via feces with only 6% of the parent compound being excreted by urine. However, the oral bioavailability was 22.5% in pigs and 30%–34% in broiler chickens [45,46]. Bioavailability (denoted as F and generally expressed as a percentage, F%), as the fraction of a drug administered by any nonvascular route that gains access to the systemic circulation [134], might therefore influence environmental occurrence. Thus, besides usage amounts, pharmacokinetics, namely bioavailability and percentage of urine excretion as parent compound could be an important and decisive factor in the occurrence of antibiotics and their metabolites in the environment.

Moreover, pharmacokinetic differences between species in the case of bioavailability can also account for reported differences in the environmental occurrence of antibiotics. For instance, for lincomycin (Rank 3) humans and swine present similar absorption values (about 50%), but different urinary excretion of the parent compound: 4%–7% in humans, 49% in dogs and 21% in pigs. Further species differences have been observed for sulfadiazine (listed 4th in Rank 3) in what respects to bioavailability. The highest absolute bioavailability was described for pigs (85%–106%) and chickens (80%), whereas lower values were observed in sheep (69%) and dairy cows (55%) [83–85].

Considering Rank 1 it is noteworthy the fact that the only antibiotic of hospital exclusive use found in the environment was vancomycin (Table 4), which is precisely the one with the highest percentage of reported urinary excretion of the parent compound (as up to 90%) and amount (nearly 1.28 tonnes). Other findings in Rank 1 are related to the 5th (clarythromycin) and 8th (azithromycin) listed antibiotics, both characterized by a low fraction of absorption in humans (50% and 38%, respectively) although both have been reported in the WWTP effluents [12]. In Portugal they were detected, mainly in hospital effluents (Table 4).

In our study, it was observed that a considerable amount of antibiotic molecules (about 36.0 tonnes of pleuromutilins, 34.0 tonnes of macrolides, 18.0 tonnes of lincosamides, 15.0 of sulfonamides and 14.0 of quinolones) were administered via drinking water and feed. Thus, presumably a large amount of these active substances may enter the environment as parent compound (biologically active) escaping to the degradation in STPs and can thus exert, first of all, a selective effect on soil and waterborne microbial community.

Top-ranked antibiotics like pleuromutilins (tiamulin, ranked 2nd and valnemulin ranked 7th), polymyxins (colistin ranked 4th) and aminoglycosides (apramycin at 10th) were not found in European

environmental surveys (Table 3). However, their occurrence in the different environmental compartments cannot be excused or neglected. Firstly the analytical methods employed in surveillance studies may not be suitable to detect them. Furthermore, the existence of bacterial resistance, observed for the aminoglycosides [28] and colistin [72] may be an indirect evidence of environmental occurrence. From our literature review it could be concluded that studies on the fate and degradation (mainly in sludge, soil and manure, biosolids) should be more deeply investigated regarding some specific antibiotics.

5. Conclusions

A ranking scheme was developed based on the assessment on Portuguese consumption data, pharmacokinetic features and environmental occurrence, as a tool to select antibiotics for future research in the field of ecosurveillance. Three lists of antibiotics were assembled: Rank 1-antibiotics only used in human medicine, Rank 2-antibiotics only used in veterinary medicine and Rank 3-antibiotics used in both practices.

An evident positive association has been observed between the developed ranking of antibiotics used in both human and veterinary medicine and their occurrence in the environment. Although this prioritization approach had been previously applied to human medicines, to the authors best knowledge, such scientifically based and pragmatic approach has never been applied in veterinary medicines in Portugal.

In conclusion, the main outcomes of this study are the following: (1) the antibiotics most used in human and veterinary medicine are those with higher potential environmental impact; (2) studies of environmental occurrence are lacking for a considerable number of human antibiotics authorized in Portugal; (3) the significance of the percentage of urine excretion as parent compound was confirmed by the fact that vancomycin was the only antibiotic of hospital exclusive use found in the environment; (4) low bioavailability might be determinant since it contributes to a higher excretion of the dosage form directly to the animals' excreta, which could explain the high levels of oxytetracycline and tylosin reported in animal manure according to the literature survey; (5) thus, besides usage amounts, pharmacokinetics could be an important and decisive factor in the occurrence of antibiotics and their metabolites in the environment.

Author Contributions

A.M. was responsible for the scientific organization of this report (mainly pharmacokinetic data) and co-wrote the first and final draft; S.D. was responsible for the scientific organization of this report (mainly environmental occurrence and used data) and co-wrote the first and final draft; R.N. Participated on the organization of human antibiotic data; H.R. and A.P. Contributed through on scientific advice; L.M. conceived and supervised this project; All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Abbreviations: DDD, defined daily dose; DGAV, Portuguese National Authority for Animal Health; EMA, European Medicines Agency; ESVAC, European Surveillance of Veterinary Antimicrobial Consumption; EU, European Union; ^{H}, Hospital exclusive use; ^{HS}, Hospital samples; INFARMED, Portuguese National Authority of Medicines and Health Products; n.d., not detectable*;* n.f., not found in our literature review; PEC, Predicted Environmental Concentration; STPs, sewage treatment plants; TMP, trimethoprim; WHO, World Health Organization; WWTPs, Wastewater treatment plants; +, found; $^{+H}$, also hospital use; % Exc. Unchanged form refers to the proportion of the molecule excreted through urine as parent compound.

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