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Hazard prioritization and risk characterization of antibiotics in an irrigated Costa Rican region used for intensive crop, livestock and aquaculture farming

Elba de la Cruz[1]*, María Luisa Fournier[1], Fernando García[2], Andrea Molina[3],
Guadalupe Chavarría[3], Margarita Alfaro[3], Fernando Ramírez[1] and César Rodríguez[2]
[1]Instituto Regional de Estudios en Sustancias Tóxicas (IRET). Universidad Nacional, Campus Omar Dengo, Heredia, 86-3000, Costa Rica
[2]Centro de Investigación en Enfermedades Tropicales (CIET) and Facultad de Microbiología. Universidad de Costa Rica, Ciudad Universitaria Rodrigo Facio, San Pedro de Montes de Oca, 2060, San José, Costa Rica
[3]Centro de Investigación en Nutrición Animal (CINA). Universidad de Costa Rica, Ciudad Universitaria Rodrigo Facio, San Pedro de Montes de Oca, 2060, San José, Costa Rica
*Corresponding Author E-mail: elba.delacruz.malavassi@una.cr

Abstract

Antibiotics alter the homeostasis of microbial communities and select for antibiotic-resistant bacteria in the wild. Thus, the accumulation of unnaturally high concentration of these substances in the environment due to their use in human activities can be regarded as a neglected form of pollution, especially in countries with agricultural-based economies. Qualitative and quantitative information on antibiotic usage in Costa Rica is scarce, hence the design and enforcement of prevention strategies and corrective measures is difficult. To address this issue, and aiming in the long run to contribute with a more rational use of pharmaceuticals in the tropics, we characterized the hazard associated with the antibiotics used during 2008 in agriculture, aquaculture, pig farming, veterinary medicine and human medicine in the major irrigation district of Costa Rica. Hazard indicators were calculated based on antibiotic use and a weighted algorithm that also considered antibiotic fate, toxicity, and resistance. Moreover, hazard quotients were computed using maximum environmental concentrations reported for Costa Rican surface waters and predicted no effect concentrations for aquatic organisms. The number of antibiotics used in the ATID during the study were n = 38 from 15 families. Antibiotic consumption was estimated at 1169-109908 g ha^{-1} $year^{-1}$ and, distinctively, almost half of this figure was traced back to phenicols. Tetracyclines, with a particular contribution of oxytetracycline, were the most widely used antibiotics in agriculture and veterinary medicine. Oxytetracycline, florfenicol, chlortetracycline, sulfamethoxazole, erythromycin, ciprofloxacin, enrofloxacin, sulfamethazine, trimethoprim and tylosin, in that order showed the highest hazard indicators. Moreover, hazard quotients greater than 1 were calculated for oxacillin, doxycycline, oxytetracycline, sulfamethazine, and ciprofloxacin. Studies dealing with the ecotoxicology of tetracyclines, sulfonamides and quinolones, as well as surveys of phenicol resistance among environmental bacteria, should be prioritized in Costa Rica.

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Introduction

Antibiotics, which are low-molecular weight molecules produced by various species of fungi and bacteria, cause diverse concentration-dependent effects on microorganisms. At unnaturally high concentrations, antibiotics inhibit bacterial replication or kill bacterial cells through lysis or oxidative stress; properties that were soon after their discovery exploited by the pharmaceutical industry and later on by animal producers to design drugs and animal growth promoters (Vidaver, 2002; WHO, 2012). These substances are nowadays recognized as the most significant therapeutic breakthrough in the history of medicine (Levy, 1992) and due to their successful application in medicine and farming; hundreds of tons of these compounds are consumed every year around the world (Wise, 2002).

Although antibiotic production is a frequent characteristic of environmental microorganisms and antibiotic producers contain numerous antibiotic-resistant genes in their genomes, the minute amount of antibiotics to which environmental bacteria are naturally exposed serve signaling functions of mutual benefit rather than inhibitory roles. (D'Acosta *et al*., 2006; Davies, *et al*., 2006; Fajardo *et al.,* 2008; Fajardo *et al.,* 2009; Ratcliff and Denison, 2011). Consequently, the ongoing accumulation of antibiotics in environmental compartments has the potential to alter the ecology of microbial communities and constitutes a neglected form of pollution (Daughton and Ternes, 1999).Several sources of antibiotics in the environment have been identified, though it is accepted that the excretions of treated people or animals, either through urban or agricultural routes, are their foremost source (Hirsch *et al.,* 1999; Segura *et al.,* 2009).

Another undesired consequence of the massive release of antibiotics into the environment is the emergence and maintenance of microbial resistance to chemotherapeutic agents. Even extremely low concentrations of clinically relevant antibiotics select for resistant bacteria (Gullberg *et al*., 2011) and this process is taking place at such an accelerated pace that microbial resistance is currently considered by the World Health Organization as a major global public health (Smith and Coast, 2002).

As to the effects of antibiotics on aquatic biota, algae and microalgae are more sensitive to these substances than crustaceans and fish, with concentrations below $100 \mu g\ l^{-1}$ being sufficient to inhibit the growth of the former two groups of organisms (Kümmerer, 2009; Yang *et al.,* 2008). Exposure of early life stages of *Daphnia* and *Artemia* species to antibiotics from different families has been linked to adverse reproductive effects (Kümmerer, 2009), while neomycin, tiamulin, enrofloxacin, and chlortetracycline have moderate to low acute toxicity toward species such as *Daphnia magna*, *Oryzias latipes and Lepomis machrochirus* (Park and Choi, 2008). These examples illustrate that antibiotics are toxic to non-target organisms in aquatic environments.

Although a prescription is required in Costa Rica to obtain antibiotics, these drugs are commonly used in productive activities without adequate technical supervision and -in some cases- without full consideration of the environmental and sanitary consequences linked to their application. Moreover, little is known in the country in relation to the amounts of antibiotics that are consumed in crop and animal farming. To address this worrisome issue, and using the aquatic environment as a model, we approximated the consumption of agricultural, veterinary, and human antibiotics during 2008 in a major irrigation district of the country and calculated hazard indicators (HI) and hazard quotients (HQ) for the antibiotics identified. This information provides a framework for future investigations and community interventions aiming to rationalize the use of antibiotics in the tropics.

Materials and Methods

Study area : The present study was conducted in an irrigation district located at the middle and lower part of the Tempisque River basin in northwestern Costa Rica (Arenal-Tempisque Irrigation District; ATID; Fig. 1). This region is characterized by more than 5 months of drought per year and the lowest precipitation levels in the country. Consequently, nearly 28000 ha in the ATID are irrigated through a series of channels that mobilizes $70m^3\ sec^{-1}$ of water released by the Arenal hydroelectric plant (Jiménez-Ramón and González-Jiménez, 2001). Since water enlarges the geographic distribution of antibiotics, the ATID was attractive for this purposes because it is subject to flooding during the rainy season and also because it is interconnected by a series of channels, rivers, and lakes. In addition, swine producers and aquaculture farmers in the ATID use medicated feed (Gutiérrez *et al*., 2010).Farmlands in the ATID are dedicated mainly to the production of sugarcane, rice, melon, watermelon, extensive beef cattle and intensive freshwater aquaculture of tilapia from the species, *Oreochromis nilotica* and *O. aurea*. These activities, together with the production of Pacific white shrimp (*Litopenaeus vannamei*) near mangroves, influence the inner part of the Gulf of Nicoya (Fig. 1). Some of the technified swine farms included in this study obtain water from exogenous sources, but their effluents do impact the ATID.

Calculation of environmental hazard indicators (HI) : Antibiotic usage patterns was observed in agriculture, veterinary, and human medicine in 2008 (U) based onconsultations to local farmers and records of antibiotic prescription in clinics and hospitals within the ATID. Representatives of about 70% of the pig producing farms and of 33%, 10%, and 30% of the ATID area dedicated to melon and watermelon, rice and aquaculture production, respectively, were interviewed personally. Thereafter, published data and employed scoring procedures were consulted to rank the antibiotics identified in U regarding their environmental fate (F) and toxicity to aquatic biota (T). These were complemented with an extensive analysis of resistance reports (R) to the antibiotics identified in U in livestock farms, livestock products, and aquatic ecosystems mainly from Mesoamerica and to a smaller extent from North and South America and other world regions (Weber *et al*., 1994; Bullock and Herman, 1998; WHO, 1998; JETACAR, 1999; Arcanglioli *et al*., 2000; Schmidt et al., 2001; Tendencia and de la Peña, 2001; Torres *et al*., 2001; White *et al*., 2001; VDD, 2002; Boxall *et al*., 2004; Burgos *et al*., 2005; Gebreyes and Thakur, 2005; Kumar *et al*., 2005; Rivera-Tapia and Cedillo-Ramírez, 2005; Tzoc *et al.*, 2005; Belém-Costa and Cyrino, 2006; Paniagua *et al*., 2006; Pringle *et al*., 2006; Vieira, 2006; Araya-Fonseca *et al*., 2007; Rodríguez *et al.*, 2007; Rodríguez-Rodríguez *et al.,* 2007; Sapkota *et al.,* 2007; Hernández-Divers *et al*., 2008; Hoa *et al*., 2008; Zhao *et al*., 2008; Alali *et al*., 2009; Chénier and Juteau, 2009; Fernández-Delgado and Suárez, 2009; OPS, 2009; Roberts *et al*., 2009; Santiago *et al*., 2009; Amábile-Cuevas, 2010; Bhakta and Munekage, 2010; Blackburn *et al*., 2010; Costa *et al*., 2010; Knapp *et al*., 2010; Rebouças *et al*., 2011; Amaya *et al*., 2012). To express U as grams of active ingredient ha^{-1} year^{-1}, size of the farms and aquaculture ponds included in ATID, area of the cities and districts serviced by the clinics mentioned above (INEC, 2009), the number of production cycles per year, and the quantity, frequency, and reason of use of each antibiotic were investigated. It was assumed that each pig occupied 2x10^{-4} ha. To quantitatively appraise F, the antibiotics were differentiated with regard to their water solubility, soil mobility, interface water-sediment and soil persistence, and bioaccumulation potential using the scoring algorithm presented in Table 1 and information taken from diverse sources (NADA, 1981; Halling-Sørensen *et al*., 1998; Halling-Sørensen *et al.,* 2000; Tolls, 2001; Jones *et al*., 2002; Koschorreck *et al*., 2002; Castiglioni *et al*., 2004; Thiele-Bruhn and Aust, 2004; Sarmah *et al*., 2006; Ter Laak *et al*., 2006; Ebner, 2007; Holmes *et al*., 2007; Kim and Carlson, 2007; NADA, 2007; Boxall, 2008; Kim *et al*., 2008; Lee *et al*., 2008; Merck, 2008; Park and Choi, 2008; Drug Bank, 2012; HSDB, 2012; PEIAR, 2012; Footprint, 2011). These parameters were chosen because a substance characterized by high water solubility, high soil mobility, long environmental persistence, and high bioaccumulation potential has a greater probability of reaching aquatic systems and exposing the biota (Walker *et al.,* 2001). T values for each antibiotic were determined using half maximal Effective Concentrations (EC$_{50}$) or No Observed Effect Concentrations (NOEC) reported for fish, crustaceans, and algae (Halling-Sørensen *et al*., 1998; Halling-Sørensen *et al*., 2000;

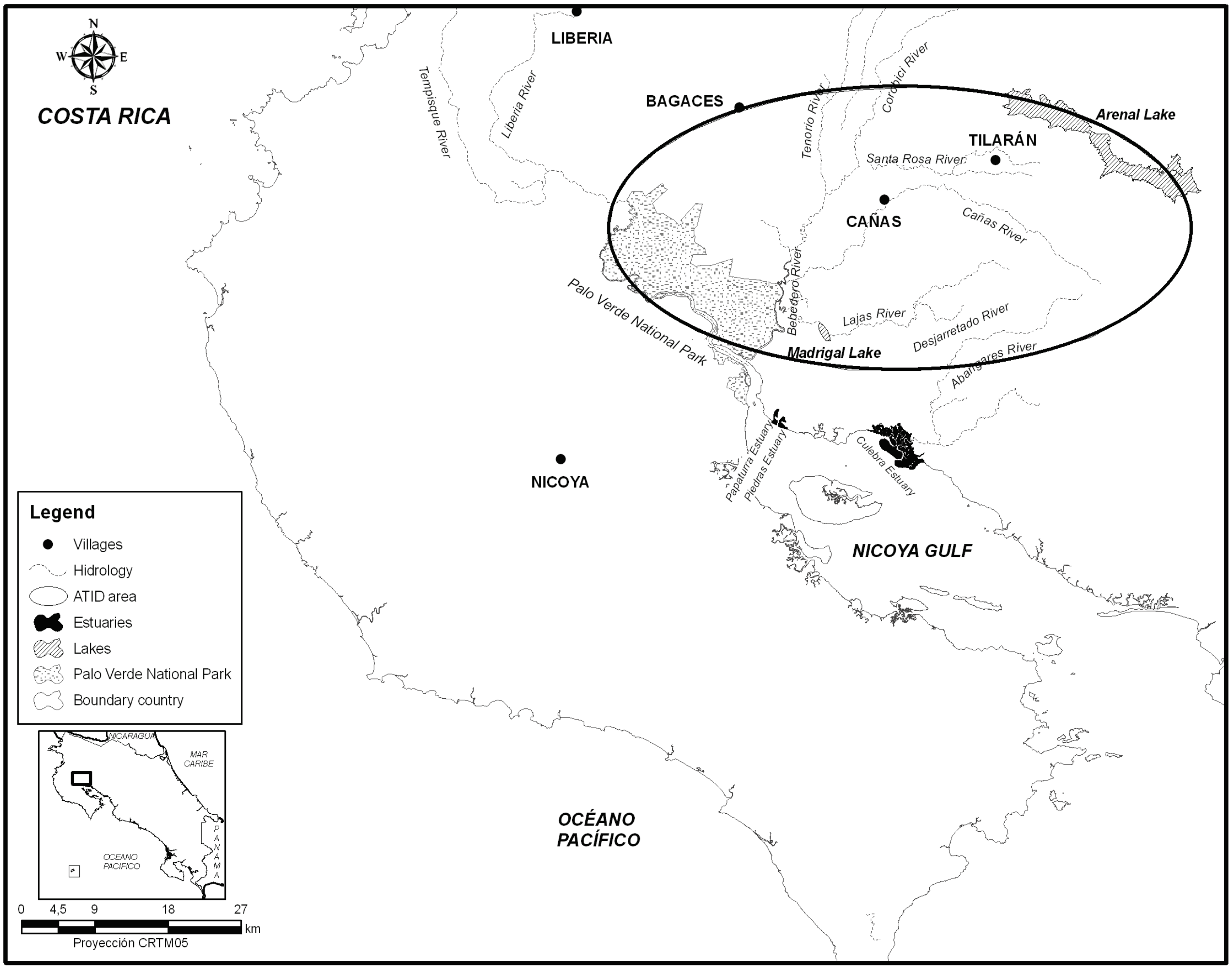

Fig. 1 : Geographical localization of the Arenal-Tempisque Irrigation District (ATID)

Holten Lützhøft *et al*., 2000; Wollenberger *et al*., 2000; Koschorreck *et al*., 2002; Reilly, 2002; Läkemedelsverket, 2004; NADA, 2004; Kumar *et al*., 2005; Carlsson *et al*., 2006; Hernando *et al*., 2006; Ter Laak *et al.*, 2006; Kim and Aga, 2007; Boxall, 2008; Kim *et al*., 2008; Merck, 2008; Park and Choi, 2008; Lai *et al.*, 2009; Yang *et al.*, 2008; Li and Randak, 2009; Footprint, 2011; Idexx, 2011; Nufarm, 2012) and the classification procedure detailed in Table 2. This table also includes the categorization procedure used in the calculation of R. The above four indicators were transformed into HI using the weighted formula: HI = (U+F+T+R)/4. In this regard, antibiotics with high HI are more likely to reach and contaminate aquatic ecosystems.

Risk characterization : Maximum environmental concentrations (MEC) reported for Costa Rican surface waters (Sponberg *et al.,* 2011), lowest Predicted No Effect Concentrations (PNEC) calculated with acute and/or chronic toxicity data for fish, algae and *Daphnia*, and assessment factors (European Commission Joint Research Center, 2003) were used to calculate Hazard Quotients. Antibiotics for which HQ>1 were obtained deserve further attention from the standpoint of environmental conservation.

Table 1. Criteria used for estimation of the environmental fate (F) of the antibiotics used in the Arenal-Tempisque Irrigation District during 2008

Characteristic	Score	Categorization
Water solubility ($mg\ l^{-1}$)	0	Low (L) < 50
	0.5	Moderate (M) 50-500
	1	High (H) > 500
Persistence in soil, water/sediment interphase, and water (DT_{50} in days)	0	Low (L) < 30 (soil and water/sediment) or <1 (water)
	0.25	Moderate (M) 30-100 (soil and water/sediment) or 1-14 (water)
	0.50	High (H) 100-365 (soil and water/sediment) or 15-30 (water)
	1	Extreme (E) > 365 (soil and water/sediment) or > 30 (water)
Soil mobility (K_{oc} in $l\ kg^{-1}$)	0	Immobile (IM) > 4000
	0.25	Low (L) 500-4000
	0.5	Moderate (M) 75-500
	0.75	High (H) 15-75
	1	Extreme (E) < 15
Bioaccumulation potential (log K_{ow})	0	Low (L) < 2.7
	0.5	Moderate (M) 2.7-3,0
	1	High (H) > 3.0

Table 2 : Criteria used for estimation of acute and chronic toxicity to fish, crustaceans, and algae (T) and of the magnitude of microbial resistance (R) of the antibiotics used in the Arenal-Tempisque Irrigation District during 2008

Parameter[a]	Score	Categorization
Toxicity to fish and crustaceans (EC_{50} or NOEC $mg\ l^{-1}$)	0.25	Low (L) >100
	0.5	Moderate (M) 10-100
	0.75	High (H) 1-10
	1	Extreme (E) < 1
Toxicity to algae, cyanobacteria, and aquatic plants(EC_{50} or NOEC $mg\ l^{-1}$)	0.25	Low (L) >10
	0.5	Moderate (M) 0.1-10
	0.75	High (H) 0.01-0,1
	1	Extreme (E) < 0.01
Resistance reports in livestock farms and animal products, or in aquatic ecosystems from Mesoamerica	0	None
	0.33	Positive(s) from one scenario
	0.66	Positive(s) from two scenarios
	1	Positive (s) from three scenarios

[a] Fish EC_{50} (96 hr) or NOEC for *Danio rerio, Lepomis macrochirus, Morone saxatilis, Oncorhynchus mykiss, Oryzias latipes* and *Salmo trutta.* Crustaceans EC_{50} (48 hr) or NOEC for *Acartia tonsa*, *Ceriodaphnia dubia, Daphnia magna, D. pulex, Litopenaeus vannamei* and *Thamnocephalus platyurus.* Algae and cyanobacteria EC_{50} (72 hr) or NOEC for *Anabaena flos-aquae, Isochrysis galbana, Microcystis aeruginosa, Nitzschia closterium; Pseudokirchneriella subcapitata, Tetraselmis chuii,* different species of *Scenedesmus, Chlorella* and *Closterium*.

Results and Discussion

Thirty-eight antibiotics from fifteen different families were used in ATID during the study period (Table 3). This figure exceeds previous reports from tropical regions like Thailand ($n < 13$) (Holmström *et al.,* 2003), as well as the usual number of antibiotics detected in hotspots for the accumulation of pharmaceuticals such as wastewater treatment plants (ca. 20-30) (Watkinson *et al.*2007; Sim *et al.,* 2011). Moreover, it comes close to the lowest number of antibiotics used in the hospitals from European countries with expectably large antibiotic inventories ($n = 35$) (MacKensie *et al.,* 2006).

By productive sector, antibiotic usage in the ATID was low. Similar to many other parts of the world, including developing countries such as Chile (Buschman *et al.,* 2012), Thailand and Mexico (Santiago, 2009; Gräslund, 2003), 14% and 85-89% of tilapia and shrimp producers interviewed, respectively, reported usage of florfenicol and oxytetracycline to prevent infections by *Vibrio* and *Rickettsia*. The U.S. Food and Drug Administration approves the use of these drugs in aquaculture (USFWS, 2011), but they will probably be replaced soon due to widespread and high levels of resistance in aquaculture ponds and mariculture environments around the world (Huy *et al.,* 2007; Rebouças *et al.,* 2011). In contrast to the prevailing situation in Vietnam, in other countries of Southeast Asia, and in Mexico (Santiago, 2009; Gräslund, 2003, Hoa *et al.,* 2011), no evidence of applications of sulfonamides, quinolones, or pyrimidines in the aquaculture farms were found.

Swine producers in the ATID reported usage of ten antibiotics from 9 different families, with chlortetracycline, florfenicol and tiamulin, sulfamethazine and tylosin and oxytetracycline being mentioned in 100%, 67%, 33% and 25% of their interviews, respectively. Their starter- and grower-feed, as well as feed for gestating and lactating sows, was medicated with tetracyclines, sulfonamides and penicillin. Tiamulin was also added to grower-feed; while phenicols were used for the treatment of respiratory symptoms and macrolides in sows. Other drugs mentioned by farmers from this industry were apramycin, virginiamycin, enrofloxacin, the antihelminthic fenbendazole and the leanness promoter ractopamine. Tylosin, chlortetracycline, and bacitracin were the most commonly used antimicrobials in grower-finisher animals in the USA (NAHMS, 2002), whereas tylosin led a similar listing in Canada (Dunlop *et al.,* 1998). In China, chlortetracycline was the most commonly used antibiotic in the swine rearing industry (Li *et al*., 2012). On the other hand, the European Union banned the administration of antibiotics and related drugs to livestock for growth promotion purposes in 2006 and the FDA passed in 2012 a rule that obliges farmers and ranchers to obtain prescription from veterinarians before using antibiotics in farm animals (FDA, 2012).

To combat *Pseudomonas fuscovaginalis*, *Burkholderia* spp., *Xanthomonas oryzae*, and *Pyricularia grisea*, 79% of the rice-, 11% of the melon- and 79% of the watermelon-farmers applied streptomycin, kasugamycin and oxytetracycline, some of them in combination with copper and ammonium salts, silver citrate and quaternary ammonium compounds. Usage of these substances was common in Latin America (Vidaver, 2002; McManus *et al.,* 2002) despite of the fact that some of these antibiotics were indeed absorbed by plants (Seo *et al.,* 2010; Dolliver *et al.,* 2007).

It has been reported that the worldwide consumption of antibiotics in livestock production almost doubles that of humans (WHO, 2012; Aarestrup, 2000). As noted in Table 3, the amount of antibiotics consumed by the pig industry in the ATID (821-107310 g ha^{-1} year^{-1}) were almost three times greater than the figures calculated for aquaculture (0-1925 g ha^{-1} year^{-1}), agriculture (14.4-340 g ha^{-1} year^{-1} and human medicine (333 g ha^{-1} year^{-1}). We did not intend to address antibiotic effects or antibiotic resistance in the pig farms visited, but scientific data, together with large diversity and quantity of antibiotics consumed in Costa Rica justify a closer examination and monitoring of this sector by environmental and sanitary authorities.

Nearly 50% of the 1169-109908 g ha^{-1} year^{-1} of antibiotics used in the ATID during the study period corresponded to phenicols (0-45947 g ha^{-1} year^{-1}), which were employed for most part in swine production and to a much lesser extent in aquaculture. This result is distinct because tetracyclines and sulfonamides often dominate consumption figures in many other places (Du and Liu, 2012). Though phenicols find little use in human medicine in Costa Rica, their observed high consumption in the ATID is worrisome. It has been suggested that Gram-positive bacteria, including several species of the human and animal pathogen *Staphylococcus* (Kehrenberg and Schwarz, 2000) become resistant to phenicols by acquisition of the gene *cfr* (chloramphenicol-florfenicol resistance). This gene, which is often located on mobile plasmids whose mobilization seems to be influenced by the use of florfenicol in the veterinary industry (Schwarz *et al.,* 2000; Schwarz *et al.,* 2004), confers resistance to phenicols, pleuromutilins, and oxazolidinones like linezolid, a last resource antibiotic used for the treatment of invasive infections caused by methicillin-resistant *S. aureus* in humans (Arias *et al.,* 2008). Linezolid is not currently used for therapy in the Costa Rican Social Security system, but the recent isolation of linezolid-resistant *S. aureus* in clinical samples from in-patients at several hospitals in Costa Rica is of great concern. We currently have no data to confirm that these linezolid-resistant *S. aureus* strains are indeed *crf*$^+$, although it is a possibility that should be evaluated in light of the consumption of phenicols in the national livestock sector.

The second place was occupied by macrolides (23059 g ha^{-1} year^{-1}), which were followed by tetracyclines (828-19374 g ha^{-1} year^{-1}), sulfonamides (40.3-17334 g ha^{-1} year^{-1}), pyrimidines (8-3724 g ha^{-1} year^{-1}), aminoglycosides (10.8-188 g ha^{-1} year^{-1}),

Table 3 : Antibiotic usage patterns in the Arenal-Tempisque Irrigation District during 2008 (g ha^{-1} $year^{-1}$)

Antibiotic family	Antibiotic	Aquaculture[a]	Swine production	Agriculture[b]	Human health	Total use
Aminoglyco-sides	Amikacyn				1.47	1.47
	Apramycin[c]		nd[f]			nd
	Gentamycin				1.91	1.91
	Kasugamycin			0-30		0-30
	Neomycin				0,03	0.03
	Streptomycin			7.4-155		7.4-155
	Total	**0**	**0**	**7.4-185**	**3.41**	**10.8-188**
Carbapenems	Imipenem				1.08	1.08
	Total	**0**	**0**	**0**	**1.08**	**1.08**
Cephalosporins	Cefalexin				49.69	49.7
	Cefalotin				30.78	30.8
	Ceftazidime				3.83	3.8
	Ceftotaxime				32.64	32.6
	Ceftriaxone				0.03	0.03
	Total	**0**	**0**	**0**	**116.98**	**117**
Streptogramins	Virginamycin[c]		nd			nd
	Total	**0**		**0**	**0**	**0**
Phenicols	Chloramphenicol				0	0.003
	Florfenicol	nd	0-45947			>0-45947
	Total	**0**	**0-45947**	**0**	**0**	**0-45947**
Macrolides	Clarithromycin				4.45	4.4
	Erythromycin				0.01	0.01
	Spiramycin				0.03	0.03
	Tylosin		0-23059			0-23059
	Total	**0**	**0-23059**	**0**	**4.5**	**4.5-23063**
Penicillins	Amoxicillin				99.12	99.1
	Ampicillin				15.50	15.5
	Oxacillin				12,02	12.0
	Penicillin[d]		nd		14.47	>14.5
	Total	**0**	**0**	**0**	**141.1**	**141.1**
Pleuromutilins	Tiamulin[e]		0-3716			0-3716
Pyrimidines	Trimethoprim				8	8
	Total	**0**	**0**	**0**	**8**	**8-3724**
Quinolones	Ciprofloxacin				0.32	0.30
	Enrofloxacin[c]		nd			0
	Levofloxacin				0.15	0.15
	Total	**0**	**0**	**0**	**0.47**	**0.47**
Sulfonamides	Sulfadiazine				0.01	0.01
	Sulfasalazine				0.31	0.31
	Sulfamethazine[e]		0-17294			0-17294
	Sulfamethoxazole				40.02	40.02
	Total	**0**	**0-17294**	**0**	**40.3**	**40.3-17334**
Tetracyclines	Chlortetracycline[e]		821-17294			821-17294
	Doxycycline				0.07	0.07
	Oxytetracycline[e]	0-1925	nd	7.4-155		7.4-2080
	Total	**0-1925**	**821-17294**	**7.4-155**	**0.07**	**828-19374**
Lincosamide	Clindamycin				11.74	11.74
Metronidazole	Metronidazole				3.98	3.98
Glycopeptide	Vancomycin				1.62	1.62
	Total	**0-1925**	**821-107310**	**14.8-340**	**333.3**	**1169-109908**

[a] Tilapia and shrimp farming; [b] Rice, melon, and watermelon farming; [c] Used in swine production; [d] benzil-Penicillin and phenylmethyl-Penicillin were considered as Penicillin; [e] Added to animal feed; [f] nd: not determined

penicillins (141 g ha^{-1} year^{-1}) and cephalosporins (117 g ha^{-1} year^{-1}). In the same order of appearance, the most commonly used representatives of these antibiotic families were tylosin, oxytetracycline and chlortetracycline, sulfamethazine, trimethoprim, streptomycin, amoxicillin, and cephalexin, cefotaxime and cephalotin (Table 3). Other compounds, such as clindamycin, metronidazol, vancomycin, imipenem, and ciprofloxacin were also reported. These results represent general estimates, as producers could have confused or disguised information to avoid potential sanctions and also because unregistered antibiotics could be applied clandestinely. The human health sector utilized the largest number of antibiotics (n = 26) and antibiotic families (n = 12), but in rather low quantities (Table 3). The use of β-lactams was basically restricted to this sector. Four of the five families of antibiotics of critical importance for animal health and human health defined by the Organization for Animal Health and the World Health Organization were identified during the survey (quinolones, macrolides, penicillins

Table 4 : Environmental fate, toxicity and resistance of the antibiotics used in the Arenal-Tempisque Irrigation District during 2008

	Characteristics^{a,b,c}									
	Environmental fate				**Toxicity**			**Resistance**		
Antibiotic	**Water solubility**	K_{ow}	**Soil mobility**	**Persistance**	**Fish**	**Crustaceans**	**Algae and cyanobacteria**	**Mesoamerica**	**Veterinary**	**Aquatic ecosystems**
Amikacyn	H	L	nd	nd	nd	nd	nd	*$_{\text{(region)}}$	*	*
Apramycin	H	L	L	E	L	L	L-E	nd	*	nd
Gentamicin	H	L	nd	nd	L	L	nd	*$_{\text{(region)}}$	*	*
Kasugamycin	nd	nd	nd	nd	L	M	L	nd	nd	*
Neomycin	H	L	E	nd	L-M	M	M	nd	*	nd
Streptomycin	H	L	nd	nd	L	L	L-E	*$_{\text{(region)}}$	*	*
Imipenem	L	L	nd	L	nd	L	nd	*$_{\text{(region)}}$	nd	*
Cefalexin	H	L	nd	nd	nd	M	nd	nd	*	*
Cefalotin	M	L	E	H-E	nd	nd	nd	*$_{\text{(region)}}$	*	*
Ceftazidime	M	L	nd	nd	nd	nd	nd	*$_{\text{(region)}}$	nd	nd
Ceftotaxime	H	L	nd	nd	nd	nd	nd	*$_{\text{(region)}}$	nd	nd
Ceftriaxone	H	L	nd	M	nd	nd	nd	*$_{\text{(ni, mx)}}$	*	*
Virginamycin	L	nd	nd	M-H	nd	nd	nd	nd	*	nd
Chloramphenicol	H	L	M	H	L	L	L-M	*$_{\text{(region)}}$	*	*
Florfenicol	H	L	H	L-E	L	L-M	M-H	*$_{\text{(usa)}}$	*	*
Clarithromycin	L	L-H	IM	L-M	L	M-H	M-E	*$_{\text{(mx)}}$	nd	nd
Eritromycin	L	H	L	L-E	L	L-E	M-H	*$_{\text{(region)}}$	*	*
Spiramycin	L	L	IM	H-E	L	M	M-E	nd	*	nd
Tylosin	H	L-H	IM-M	L-E	L-M	L-M	M-E	nd	*	nd
Amoxicillin	H	L	L	L	L	L	L-E	*$_{\text{(region)}}$	*	*
Ampicillin	H	L	L	ND	L	L	L	*$_{\text{(region)}}$	*	*
Oxacillin	L	M-H	nd	ND	nd	nd	nd	*$_{\text{(region)}}$	*	*
Penicillin	M-H	L	M	L-M	nd	nd	E	*$_{\text{(region)}}$	*	*
Tiamulin	L	H	nd	M-H	H	M-H	M-E	nd	*	*
Trimethoprim	M	L	L	L-E	L	L-M	L-E	*$_{\text{(region)}}$	*	*
Ciprofloxacin	L-H	L-M	IM-M	L-H	L	M	L-E	*$_{\text{(region)}}$	*	*
Enrofloxacin	H	L-M	IM	M-E	L-M	L-H	M	*$_{\text{(be, mx)}}$	*	*
Levofloxacin	nd	L	nd	L-H	L	E	M	*$_{\text{(cr, ni, mx)}}$	*	*
Sulfadiazine	M	L	M-H	M-H	nd	L-M	L-M	nd	nd	*
Sulfasalazine	M	M-H	L	L-H	nd	L	nd	nd	nd	nd
Sulfamethazine	H	L	M-H	L-M	L	L	M-E	*$_{\text{(region)}}$	*	*
Sulfamethoxazole	H	L	H	L-H	L	L-E	L-E	*$_{\text{(be, ni, pa, mx)}}$	*	*
Chlortetracycline	H	L	IM-M	M	M-E	L-M	M-E	*$_{\text{(cr, pa, mx)}}$	*	*
Doxycycline	H	L	nd	nd	nd	nd	M	*$_{\text{(be, cr)}}$	*	*
Oxytetracycline	H	L	IM-M	L-E	L-M	L-E	M	*$_{\text{(cr)}}$	*	*
Clindamicin	L	L	M	M	nd	L-M	H	*$_{\text{(region)}}$	*	*
Metronidazole	H	L	H	L-M	L	L	L	*$_{\text{(cr, mx)}}$	nd	*
Vancomycin	H	L	nd	nd	nd	nd	nd	*$_{\text{(region)}}$	*	*

^{a}L: low; M: moderate; H: high; E: extreme; IM: immobile (see Tables 1-2); ^{b}ND: no data available; ^{c}region: Central America; be: Belize; cr: Costa Rica; mx: Mexico; ni: Nicaragua; USA: United States of America; Soure: refer to text section Material and methods.

and aminoglycosides) (FAO/WHO/OIE, 2007).

A number of antibiotics identified in the ATID have potential to reach and impact the water bodies (Table 4): 74% of them were moderate to highly soluble in water, 37% were moderate to highly mobile in soil, 60% were persistent in the environment and 23% had potential to bioaccumulate within the organisms (Kumar *et al.,* 2005). In descending order, the antibiotics showing the highest F estimators and therefore the highest potential to pollute aquatic systems were tylosin, florfenicol, oxytetracycline, cephalotin, enrofloxacin and ciprofloxacin (Table 5). Nine antibiotics identified in the ATID, including doxycycline, oxacillin, sulfamethazine, ciprofloxacin, oxytetracycline, trimethoprim, tetracycline, clarithromycin and sulfamethoxazole have been previously detected at concentrations between ng l^{-1} and µg l^{-1} in water bodies from

Table 5 : Hazard indicators and prioritization of the antibiotics used in the Arenal-Tempisque Irrigation District during 2008

Antibiotic family	Antibiotic	Usage (U)	Fate (F)	Toxicity (T)	Resistance (R)	Hazard indicator (HI)
Tetracyclines		0.84	0.63	0.92	1	0.85
	Oxytetracycline	0.56	0.63	0.67	1	0.72
	Chlortetracycline	0.25	0.44	0.83	1	0.63
	Doxycycline	0.16	0.25	0.17	1	0.40
	Macrolides	0.41	0.88	0.83	1	0.78
	Erythromycin	0.16	0.56	0.67	1	0.60
	Tylosin	0.25	0.88	0.67	0.33	0.53
	Spiramycin	0.16	0.50	0.58	0.33	0.39
	Clarithromycin	0.16	0.31	0.67	0.33	0.37
Sulfonamides		0.41	0.81	0.75	1	0.74
	Sulfamethoxazole	0.16	0.56	0.75	1	0.62
	Sulfamethazine	0.25	0.50	0.50	1	0.56
	Sulfadiazine	0.16	0.44	0.33	0.33	0.32
	Sulfasalazine	0.16	0.56	0.08	0.01	0.20
Aminoglycosides		0.47	0.75	0.67	1	0.72
	Streptomycin	0.19	0.25	0.50	1	0.49
	Gentamycin	0.16	0.25	0.17	1	0.40
	Apramycin	0.13	0.56	0.5	0.33	0.38
	Neomycin	0.16	0.5	0.5	0.33	0.37
	Amikacyn	0.16	0.25	0.01	1	0.35
	Kasugamycin	0.16	0.01	0.33	0.33	0.21
Quinolones		0.28	0.75	0.83	1	0.72
	Ciprofloxacin	0.16	0.63	0.58	1	0.59
	Enrofloxacin	0.13	0.63	0.5	1	0.57
	Levofloxacin	0.16	0.13	0.58	1	0.47
Phenicols		0.53	0.69	0.33	1	0.64
	Florfenicol	0.38	0.69	0.50	1	0.64
	Chloramphenicol	0.16	0.50	0.33	1	0.50
Penicillins		0.31	0.69	0.5	1	0.63
	Penicillin	0.28	0.44	0.33	1	0.51
	Amoxicillin	0.19	0.31	0.5	1	0.50
	Ampicillin	0.16	0.31	0.25	1	0.43
	Oxacillin	0.16	0.25	0.01	1	0.35
Pyrimidines	Trimethoprim	0.16	0.44	0.58	1	0.55
Pleuromutilines	Tiamulin	0.25	0.38	0.83	0.67	0.54
Cephalosporins		0.19	0.75	0.17	1	0.53
	Cefalotin	0.16	0.63	0.01	1	0.45
	Ceftriaxone	0.16	0.31	0.01	1	0.37
	Cefalexin	0.16	0.25	0.33	0.66	0.35
	Ceftotaxime	0.16	0.25	0.01	0.33	0.19
	Ceftazidime	0.16	0.13	0.01	0.33	0.16
Lincosamides	Clindamicin	0.16	0.25	0.42	1	0.46
Metronidazole	Metronidazole	0.16	0.5	0.25	0.67	0.40
Glycopeptides	Vancomycin	0.16	0.25	0.01	1	0.35
Carbapenems	Imipenem	0.16	0.01	0.08	0.67	0.23
Streptogramins	Virginamycin	0.13	0.13	0.01	0.33	0.15

Costa Rica (Sponberg *et al.,* 2011) and from other regions of the world (Hernando *et al.,* 2006). Moreover, three of the antibiotics included in top 10 list of pharmaceuticals of critical importance for the water cycle communicated by the Global Water Research Coalition (GWRC, 2008), namely sulfamethoxazole, ciprofloxacin and erythromycin, were used in the ATID during 2008.

In ascending order, 16, 47 and 60% of the antibiotics identified were moderately or highly toxic to fish, crustaceans and aquatic flora (Table 4). Regarding toxicity, the highest ranks were occupied by tiamulin and chlortetracycline followed by sulfamethoxazole, oxytetracycline, erythromycin, tylosin and clarithromycin (Table 5). Acute toxicity data for fish, crustaceans, and algae were not available for 39%, 26% and 32% of the compounds, respectively. This lack of information confirms that further research on the ecotoxicology of antibiotics is required in the tropics.

In northern Europe, environmental prioritization studies on antibiotics are mainly based on use, persistence, bioaccumulation and toxicity data (GWRC, 2008). We expanded this approach to include the occurrence of antibiotic-resistant bacteria in the environment because this trait represents an adaptation towards antibiotic pollution in human, domestic animals and wild life populations and also because antibiotic resistance represents a heavy burden to most economies and health systems of the world (Maragakis *et al*., 2008). Reports of resistant bacteria were available for all of the antibiotics detected in the ATID, and when this search was restricted to the Mesoamerican region, reports for all structural classes -except for the streptogramins and pleuromutilins- were found (Table 4). Up

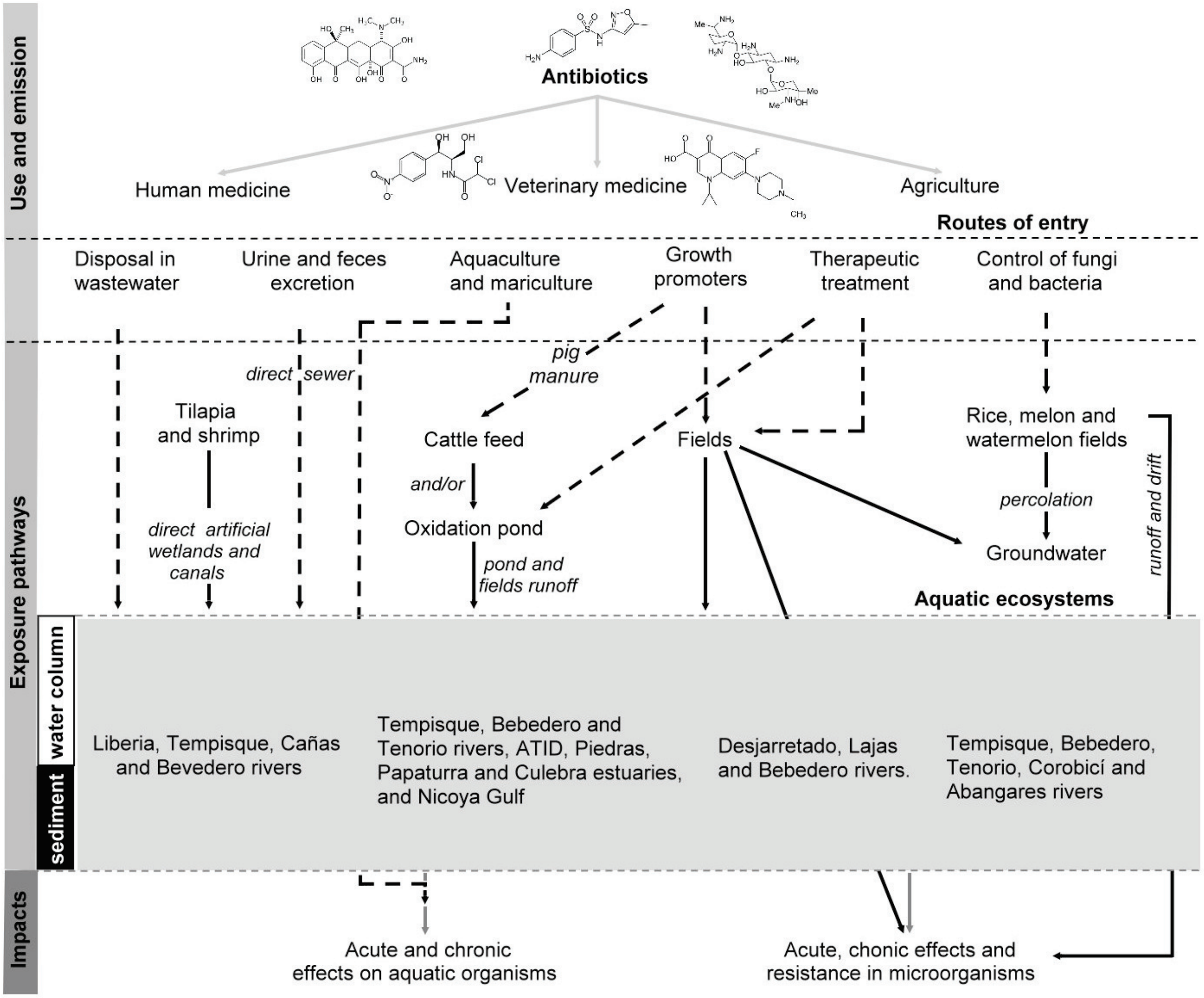

Fig. 2 : Routes of entry, foreseen exposure pathways, and impacts of agricultural-, veterinarian- and human-antibiotics used in the Arenal-Tempisque Irrigation District during 2008.

Table 6 : Hazard quotients calculated for the antibiotics used in the Arenal-Tempisque Irrigation District during 2008

Antibiotic	Maximum surface water concentration reported in Costa Rica[a](MEC; $\mu g\ l^{-1}$)	Predicted No Effect Concentration (PNEC; $\mu g\ l^{-1}$)	Hazard Quotient (MEC/PNEC)
Oxacillin	7.571	0.6 (algae)	12.6
Doxycycline	73.722	30 (algae)	2.45
Oxytetracycline	0.428	0.2 (algae)	2.1
Sulfamethazine	1.626	0.9 (algae)	1.9
Ciprofloxacin	0.740	0.4 (algae)	1.8
Clarithromycin	0.063	0.2 (algae)	0.3
Trimethoprim	0.122	0.4 (algae)	0.3
Sulfamethoxazole	0.056	0.2 (algae)	0.3
Tetracycline	0.093	9 (algae)	0.1
Clindamycin	0.008	3.0 (*Daphnia*)	0.003

[a] Spongberg *et al.* 2011

to 87% of these reports were derived from veterinarian environments and animal products, while 93% stem from aquatic environments. No reports of resistance to carbapenems and metronidazole in the former group of habitats or of resistance to streptogramin among aquatic bacteria were found. In addition to antibiotics, farmers of the ATID reported the use of disinfectants and biocides that may co-select for mobile genetic elements carrying antibiotic resistance genes (Gilbert and McBain, 2003; Davies and Davies, 2010).

The antibiotic families linked to the highest HI were tetracyclines, macrolides, sulfonamides, aminoglycosides, quinolones, phenicols and penicillins (Table 5). A similar ranking of active ingredients revealed elevated HI for oxytetracycline, florfenicol, chlortetracycline, sulfamethoxazole, erythromycin, ciprofloxacin, enrofloxacin, sulfamethazine, trimethoprim and tylosin (Table 5). HQ > 1 were obtained for penicillins (oxacillin = 12.6), tetracyclines (doxycycline = 2.4, oxytetracycline = 2.1), sulfonamides (sulfamethazine = 1.9) and quinolones (ciprofloxacin = 1.8). Therefore, four of the fifteen antibiotic families identified in the ATID might pose a significant risk for the aquatic ecosystem (Table 6). These antibiotics have also been prioritized in Korea (Kim *et al.,* 2011), China and Europe (Park and Choi, 2008).

Based on field trips and interviews, the routes of entry, exposure pathways and potential impacts of the antibiotics identified in the ATID are summarized in Fig. 2. Mariculture effluents reach Papaturro, Piedras, Culebra and Gulf of Nicoya estuaries. Fish farm effluents are disposed into artificial wetlands, rice paddy fields, sugar cane plantations and the Tenorio, Tempisque, Bebedero and Cañas rivers. These rivers are the main receptors of agricultural effluents along with the rivers Corobicí and Abangares and the drainage channels of the irrigation district. Pig manure is used for cattle feeding, discharged in oxidation ponds, or deposited together with other pig effluents in neighboring fields. From there, residues may runoff to the Desjarretado river and contaminate the Lajas and Bebedero rivers. Human waste, which likely contains antibiotics and antibiotic-resistant bacteria, also might reach the Liberia, Tempisque, Cañas, and Bebedero rivers.

Though it has been difficult to establish a precise quantitative relationship between the frequency of resistance to a defined antibiotic and the volume of drug use (Sepälä *et al.,* 1997; Gao *et al.,* 2012), data of the present study supports the concept that the use of antibiotics in agriculture has a greater impact on the dispersion of resistance and on environmental degradation than their use in the human health sector. Consequently, the authors believe that Costa Rica requires explicit water quality standards, a surveillance system of antibiotic usage outside clinical settings, and monitoring programs aimed at reducing the environmental burden of these biologically active compounds.

Our approach proved to represent a useful tool to summarize antibiotic use in a tropical Mesoamerican productive area and to prioritize their hazards. The information presented is of relevance not only due to its potential to influence decision takers and policy makers, but also because it provides a framework for future investigations and community interventions aiming to rationalize the use of antibiotics in tropical countries. In Costa Rica, studies dealing with the ecotoxicology of tetracyclines, sulfonamides and quinolones should be emphasized. Moreover, microbial resistance to phenicols in the environment should be followed closely.

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