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In silico evaluation of interactions between antibiotics in aquaculture and nuclear hormone receptors

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ABSTRACT: Antibiotics have been commonly used as antimicrobial agents in the process of aquaculture worldwide. However, very few studies are available on the endocrine disruption-related health risks brought about by antibiotic residues from human consumption of aquatic products. Nuclear hormone receptors (NHRs) could mediate many endocrine-disrupting activities. Therefore, in the present study, a reverse docking method was used to predict the direct binding interactions between 16 NHR conformations and 15 common antibiotics in aquaculture, thereby determining their potential endocrine-disrupting risks. To reach a compromise between the extremely scarce experimental data and an urgent need for distinguishing antibiotics of high concern with potential food-borne endocrine-disrupting risks in aquaculture, a risk-ranking system was then developed based on a comprehensive risk score for each category of antibiotics, which was the sum of the products of endocrine-disrupting potential coefficients and annual usages of antibiotics in aquaculture. The results indicated that 15% of 224 docking simulations showed a relatively high probability of binding. Sulfonamides seemed to possess the greatest endocrine-disrupting potential. The antagonistic conformation of the androgen receptor was the most susceptible NHR conformation. The rank orders of the endocrine-disrupting risk of different categories of antibiotics varied greatly from country to country, which were significantly affected by the annual usage. These findings pose questions regarding public health and safety associated with human consumption of antibiotic-containing aquatic products. In addition, we provide an approach to rank antibiotics for a specific country or region, with respect to their potential endocrine-disrupting activity, that can be used to inform regulation and prioritize experimental verification.

KEY WORDS: Aquaculture antibiotics · Endocrine disruption · Nuclear hormone receptor · Inverse docking · Risk ranking

1. INTRODUCTION

Industrial aquaculture is a rapidly growing industry (Little et al. 2016). It is estimated that global fish production in 2018 was about 179 million t, of which aquaculture production accounted for 52% (FAO 2020). Many countries have invested heavily in aquaculture in order to meet people's demand for high-quality and affordable nutrients such as protein and fat (Boyd et al. 2020). In order to improve the sur-

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vival rate of farmed aquatic organisms, antimicrobial drugs are often used in large-scale aquaculture to deal with aquatic animal diseases caused by pathogenic bacteria. The global antimicrobial consumption in aquaculture was estimated at 10 259 t (95% uncertainty interval [UI]: 3163–44 727 t) in 2017, and is expected to increase by 33% to 13 600 t (UI: 4193–59 295) in 2030 (Schar et al. 2020). Antibiotics have been widely used in the process of nursery and breeding in aquaculture. They are mainly used in the treatment and prevention of intestinal parasites and systemic diseases (Serrano 2005) and mixed in the feed as growth promoters in some countries e.g. in Egypt, to promote fish growth (Reda et al. 2013). According to their mechanism of action and chemical structure, the antibiotics commonly used in aquaculture can be classified into chemical groups, of which the most common categories include sulfonamides, fluoroquinolones, β-lactams, tetracyclines, amphenicols and macrolides (Kümmerer 2009). Worldwide antibiotic consumption has been estimated approximately to lie between 100 000 and 200 000 t annum^{-1} (Wise 2002, Kümmerer 2003), with about half being used for veterinary purposes (Sarmah et al. 2006). However, the amount of antibiotics used in aquaculture worldwide is difficult to estimate because different countries vary considerably with respect to their registration systems, not to mention that few countries monitor this (Heuer et al. 2009, Romero et al. 2012). Even in the many countries that have a registration system, antibiotic usage patterns may be very different. For example, annual production of antibiotics in China was about 210 000 t, of which 46% was estimated to be used in livestock and poultry feeds (Wang & Ma 2008). In addition, it was estimated that the use of antimicrobial agents in aquaculture accounted for nearly 15% of the total veterinary use in China (Wu 2019). Available data indicate that, for food-producing animals in China in 2013, the annual total usage amounts for individual antibiotics including sulfonamides, fluoroquinolones, β-lactams, tetracyclines, amphenicols and macrolides were 5491 (19% of total antibiotics usage), 9607 (<33%), 128 (0.4%), 1567 (5%), 10 000 (34%) and 2526 (9%) t, respectively (Zhang et al. 2015). In the USA in 2018, the corresponding usage amounts were 279 (5% of total antimicrobial agents usage), 23 (<1%), 763 (13%), 3974 (66%), 56 (1%) and 478 (8%) t, respectively, which varies greatly from China (USFDA 2019). The published literature and government reports have been searched; however, no contemporary antibiotic usage data could be found for aquaculture for China or the USA. The large variation in total usage and proportion of each antibiotic may be attributed to difference in period, farming scale, farming geographic area, types of aquatic bacterial pathogens, antibiotic price and regulatory standards between countries (Miranda et al. 2018).

The use of antibiotics in aquaculture inevitably brings potential hazards to human health. Food-borne exposure of antibiotics in humans may lead to allergic reactions, toxic effects, changes in the intestinal microflora and increased antibiotic resistance (Mo et al. 2017). In addition, increasing evidence suggests that antibiotics can lead to endocrine disorders, although they are not typical endocrine-disrupting chemicals (EDCs) (Kim et al. 2017, Park & Kwak 2018, Yu et al. 2020). For example, antibiotics such as erythromycin, oxytetracycline, sulfathiazole and chlortetracycline were shown to interfere with steroidogenic gene expression and hormone production in H295R cells (Gracia et al. 2007, Ji et al. 2010). Adverse effects on spermatogenesis and spermatozoal function have been well documented in mammals (Wallach et al. 1991). Yu et al. (2020) found that long-term exposure to low concentrations of oxytetracycline could disrupt the thyroid system and affect the growth and development of zebrafish (Perkins et al. 2013).

In the endocrine system, nuclear hormone receptors (NHRs) play key roles in maintaining endocrine homeostasis (Hall & Greco 2019). However, very few studies have been conducted to investigate the direct binding and interaction between NHRs and antibiotics with potential endocrine-disrupting activity. Cefuroxime was determined to be estrogen receptor (ER) agonistic by stable transcriptional activation assays in VM7Luc4E2 cells (Lee et al. 2019). Rifampicin was found to be a nonsteroidal ligand and activator of the human glucocorticoid receptor using amino acid mutation and transient transactivation assays (Calleja et al. 1998). Sulfadiazine was found to be able to bind to ERα by the use of a molecular docking method (Bowker 2015).

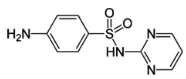
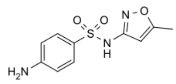
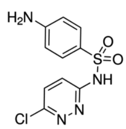
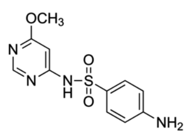
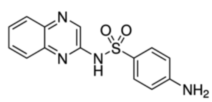
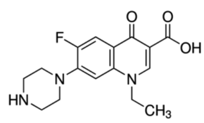
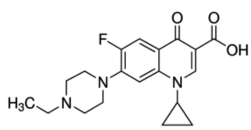
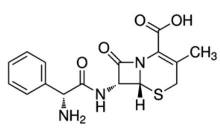
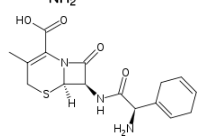
Biochemical and cell-based experimental approaches, such as transactivation assays and radioligand binding assays, are time-consuming and cost-intensive. More cost-effective computational methods have been approved as alternatives to identify novel agonists or antagonists of receptors (Krewski et al. 2010, Plošnik et al. 2015), including quantitative structure–activity relationship (QSAR) modeling, molecular dynamic (MD) simulation and docking (Ruiz et al. 2017). Among them, a reverse-docking approach has been widely used to predict binding affinity scores between a library of biological macromolecules and specific small molecules of interest, such as drugs, herbicides, ingredients of cosmetic products, bisphenol-A analogs and organophosphate esters (Schapira et al. 2003, Do et al. 2005, Grinter et al. 2011, Kharkar et al. 2014, X. Wang et al. 2017, Usman & Ahmad 2019, Wang et al. 2020). This approach has been demonstrated to be more cost-effective and more efficient for identifying potential biological targets of compounds with relatively high accuracy, sensitivity and specificity than *in vitro*/*in vivo* experimental approaches (Schapira et al. 2003, Do et al. 2005, Grinter et al. 2011, Wang et al. 2020). Thus, in the present study, we predicted the capacities of 15 antibiotics commonly used in aquaculture to bind with 16 different human NHR conformations using a reverse-docking simulation model and ranked the health risks posed by various categories of antibiotics by combining their endocrine-disrupting potential with their annual usage data.

2. MATERIALS AND METHODS

2.1. Antibiotics tested

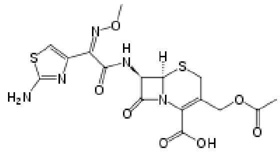
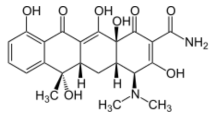
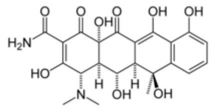
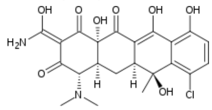
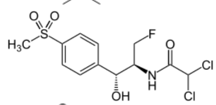
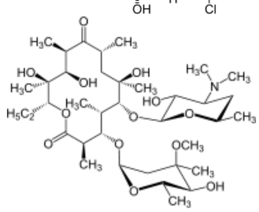
Table 1 lists the chemical information for the 15 antibiotics (6 categories) included in the present study: sulfonamides (sulfadiazine, sulfamethoxazole, sulfachloropyridazine, sulfamonomethoxine and sulfaquinoxaline), fluoroquinolones (norfloxacin and enrofloxacin), β-lactams (cefalexin, cefradine and cefo-

Table 1. Chemical information for the widely used aquaculture antibiotics tested in the present study. CAS: Chemical Abstracts Service

Antibiotic	Abbreviation	CAS number	Structure	Formula	Relative molar mass
Sulfonamides					
Sulfadiazine	SDZ	68-35-9		$C_{10}H_{10}N_4O_2S$	250.28
Sulfamethoxazole	SMX	723-46-6		$C_{10}H_{11}N_3O_3S$	253.28
Sulfachloropyridazine	SCP	80-32-0		$C_{10}H_9ClN_4O_2S$	284.72
Sulfamonomethoxine	SMM	1220-83-3		$C_{11}H_{12}N_4O_3S$	280.30
Sulfaquinoxaline	SQX	59-40-5		$C_{14}H_{12}N_4O_2S$	300.34
Fluoroquinolones					
Norfloxacin	NFX	70458-96-7		$C_{16}H_{18}FN_3O_3$	319.33
Enrofloxacin	EFX	93106-60-6		$C_{19}H_{22}FN_3O_3$	359.39
β-lactams					
Cefalexin	CLX	15686-71-2		$C_{16}H_{17}N_3O_4S$	347.39
Cefradine	CRD	38821-53-3		$C_{16}H_{19}$N3O4S	349.41

(*continued on next page*)

Table 1 (continued)

Antibiotic	Abbreviation	CAS number	Structure	Formula	Relative molar mass
β-lactams					
Cefotaxime	CTX	63527-52-6		$C_{16}H_{17}N_5O_7S_2$	455.47
Tetracyclines					
Tetracycline	TC	60-54-8		$C_{22}H_{24}N_2O_8$	444.44
Oxytetracycline	OTC	79-57-2		$C_{22}H_{24}N_2O_9$	460.43
Chlortetracycline	CTC	57-62-5		$C_{22}H_{23}ClN_2O_8$	478.88
Amphenicols					
Florfenicol	FF	73231-34-2		$C_{12}H_{14}Cl_2FNO_4S$	358.21
Macrolides					
Erythromycin	ETM	114-07-8		$C_{37}H_{67}NO_{13}$	733.94

taxime), tetracyclines (tetracycline, oxytetracycline and chlortetracycline), amphenicols (florfenicol) and macrolides (erythromycin). They were selected due to their common use in aquaculture.

2.2. Reverse-docking simulation

Reverse-docking simulation was conducted using the open-source software Endocrine Disruptome (Kolšek et al. 2014), with a free access interface available at http://endocrinedisruptome.ki.si/. The antibiotics were introduced into the software by the simplified molecular input line entry system (SMILES) on the website prediction interface. Then the antibiotic molecules were docked via AutoDock Vina to 16 integrated and well-validated crystal structures of 12 different human NHRs, including 12 agonistic conformations: androgen receptor (AR), ERα/β, glucocorticoid receptor (GR), liver X receptors α and β (LXRα/β), peroxisome proliferator-activated receptors α, β and γ (PPARα/β/γ), retinoid X receptor α (RXRα) and thyroid hormone nuclear receptors α and β (TRα/β); and 4 antagonistic conformations: AR antagonist (AR *an*), ERα antagonist (ERα *an*), ERβ antagonist (ERβ *an*), GR antagonist (GR *an*). The score of the binding affinity between each antibiotic molecule with the individual receptors was calculated. Four probability binding classes, i.e. high, medium-high, medium and low probability, were defined per conformation based on 3 sensitivity thresholds, which were approximately equal to 0.25, 0.5 and 0.75, respectively (Kolšek et al. 2014). Sensitivity can be interpreted as a true-positive rate. To a certain extent, the 4 classes can be considered as the potential strength of endocrine-disrupting activity.

2.3. Risk scoring and ranking

A risk-scoring and -ranking system was developed to distinguish the hierarchical health risks of different antibiotics brought by human consumption of aquatic products (i.e. food-borne health risks). The endocrine-disrupting potential coefficients of 1, 0.75, 0.5 and 0 were assigned to different binding probabilities from high to low. The sum of the coefficients of each NHR for individual antibiotic was multiplied by its corresponding usage in aquaculture to get a comprehensive score of health risk for ranking the antibiotics.

3. RESULTS

3.1. Antibiotics with potential endocrine-disrupting activity

The scores of direct binding affinity between the 15 antibiotic molecules and NHRs were predicted by reverse docking and are listed in Table 2. Erythromycin, whose relative molecular mass is >500, was basically unable to bind NHRs due to its large structure, and thus the following discussion is conducted based on the other 14 antibiotics. Among the 224 binding interactions, no binding was high probability, while about 15% had medium-high to medium probabilities. The remaining 85% were low probability. For sulfonamide antibiotics, 21% of the bindings had medium-high to medium probabilities, which was higher than that for all the antibiotics, i.e. 15% mentioned in the previous sentence. Moreover, sulfonamides were the only category of antibiotics which showed medium-high probability bindings to NHRs in the present study. For fluoroquinolone antibiotics, 22% of their bindings with NHRs displayed medium probability. For β-lactams, tetracyclines and amphenicols antibiotics, nearly 90% or higher showed low probability of binding. Thus, sulfonamide antibiotics were likely to possess the greatest endocrine-disrupting potential.

3.2. Vulnerable conformations of NHRs

As shown in Table 2, the antagonistic conformation of AR was the most vulnerable conformation, followed by the GR and TRs, i.e. they were combined with more antibiotics with higher binding probability. Almost all sulfonamides, fluoroquinolones, β-lactams and amphenicols except for tetracyclines studied in the present study could bind to the antagonistic conformation of AR with medium or medium-high probability.

3.3. Risk ranking

Based on the binding probability and the usage of different kinds of antibiotics in aquaculture, compre-

Table 2. Binding affinity scores of antibiotic molecules with 16 human nuclear hormone receptor conformations (12 agonistic and 4 antagonistic [*an*] conformations) predicted by reverse docking. The more negative the value, the higher the score. Gradation of binding affinity scores was done based on the probability of binding. Probability binding classes are color-coded: purple: medium-high probability; yellow: medium probability; and green: low probability. AR: androgen receptor; ERα/β: estrogen receptors α and β; GR: glucocorticoid receptor; LXRα/β: liver X receptors α and β; PPARα/β/γ: peroxisome proliferator-activated receptors α, β and γ; RXRα: retinoid X receptor α; TRα/β: thyroid hormone nuclear receptors α and β. Antibiotic abbreviations as in Table 1

Antibiotic	AR	AR *an*	ERα	ERα *an*	ERβ	ERβ *an*	GR	GR *an*	LXRα	LXRβ	PPARα	PPARβ	PPARγ	RXRα	TRα	TRβ
Sulfonamides																
SDZ	−7.5	−7.7	−7.4	−7.6	−7.6	−7.7	−6.9	−6.8	−7.3	−7.5	−7.0	−7.1	−6.9	−7.4	−7.6	−7.5
SMX	−7.7	−7.5	−7.4	−7.6	−7.1	−7.4	−7.2	−6.7	−7.9	−7.6	−6.9	−7.2	−7.5	−7.5	−7.4	−7.5
SCP	−7.3	−7.8	−8.0	−7.8	−7.2	−7.9	−7.6	−7.1	−8.2	−8.1	−7.4	−7.6	−7.4	−7.8	−7.5	−8.2
SMM	−7.5	−7.6	−7.4	−7.8	−7.5	−7.4	−7.2	−7.0	−7.9	−7.7	−7.2	−7.4	−6.9	−7.8	−7.1	−7.5
SQX	−6.9	−8.0	−8.4	−8.6	−7.9	−8.2	−8.4	−8.1	−9.1	−9.1	−8.3	−8.5	−8.6	−8.9	−8.2	−8.7
Fluoroquinolones																
NFX	−7.2	−6.6	−7.4	−6.3	−6.6	−6.6	−8.4	−6.9	−8.7	−9.4	−7.3	−7.8	−7.7	−7.5	−8.5	−9.2
EFX	−6.3	−6.2	−7.2	−6.5	−6.0	−6.4	−8.6	−7.4	−8.2	−9.2	−6.8	−8.1	−7.8	−7.9	−6.7	−8.6
β-lactams																
CLX	−6.1	−7.2	−7.8	−6.7	−7.6	−7.1	−8.3	−8.1	−8.4	−9.1	−7.0	−8.2	−7.4	−8.3	−6.7	−8.4
CRD	−5.8	−7.0	−7.1	−7.0	−7.2	−7.0	−8.4	−7.6	−8.5	−9.2	−7.3	−8.1	−7.4	−8.7	−6.7	−8.2
CTX	−1.8	−3.1	−4.5	−5.7	−2.0	−5.8	−7.3	−7.3	−7.4	−7.7	−5.9	−7.9	−6.4	−6.4	−2.9	−4.7
Tetracyclines																
TC	3.3	1.0	−5.6	−7.4	1.0	−6.5	−5.8	−7.9	−4.1	−6.3	−4.5	−6.4	−6.7	−2.4	8.9	−0.8
OTC	3.3	1.1	−5.6	−6.9	−0.1	−6.2	−5.3	−8.0	−4.1	−6.5	−4.4	−6.5	−6.7	−1.6	9.9	−0.8
CTC	5.5	3.1	−4.9	−7.4	5.6	−6.6	−4.8	−8.3	−3.9	−5.3	−4.5	−5.1	−9.1	−2.0	13.1	5.9
Amphenicols																
FF	−6.9	−7.3	−6.4	−6.8	−7.0	−6.8	−7.5	−6.5	−8.0	−8.4	−6.7	−7.0	−6.8	−7.5	−7.2	−7.7
Macrolides																
ETM	Not simulated since relative molecular mass exceeds 500															

hensive scores of the health risks of individual antibiotics were calculated and compared to determine relative food-borne health risks. Since no data was available for aquaculture, the usage of different types of antibiotics in aquaculture is from food-producing animals including aquaculture (Table 3). This differs significantly among countries. Available data indicated that, in China, amphenicols and fluoroquinolones were the most widely used antibiotics in 2013. However, in the USA in 2018, tetracycline antibiotics were most widely used in food-producing animals. The food-borne health risks brought about by various antibiotics in production of food-producing animals vary greatly from country to country. The rank order of the health risk was fluoroquinolones > amphenicols > sulfonamides > β-lactams > tetracyclines in China, while it was β-lactams > tetracyclines > sulfonamides > amphenicols > fluoroquinolones in the USA.

Table 3. Comprehensive scores of health risk for various categories of antibiotics based on their endocrine-disrupting potential coefficient (k) and annual usage (U) in aquaculture. Data for U is from food-producing animals (China: Zhang et al. 2015; USA: USFDA 2019), since no data was available for aquaculture. For China, for each antibiotic category, the comprehensive score of health risk is equal to: $\sum_{i=1}^{n}$ [Endocrine-disrupting potential coefficient (k_i)] × annual usage (U_i). For the USA, no antibiotic-specific U data was available, thus the average of k for each antibiotic for each category was multiplied by total U to get the comprehensive score of health risk: $\sum_{i=1}^{n}$ [Endocrine-disrupting potential coefficient (k_i)] / n × total annual usage (U_{total}). n: number of antibiotics included in a category; NA: not available; ND: not determined since relative molecular weight exceeds 500. Antibiotic abbreviations as in Table 1

Antibiotic	k	Annual U (t) China in 2013	Annual U (t) USA in 2018	Health risk score China	Health risk score USA
Sulfonamides					
SDZ	1.75	1022			
SMX	1.5	311			
SCP	2.25	518	279	9581	516
SMM	1	2200			
SQX	2.75	1440			
Fluoroquinolones					
NFX	2	4427	23	16624	40
EFX	1.5	5180			
β-lactams					
CLX	1.5	128			
CRD	1.5	NA	763	192	763
CTX	0	NA			
Tetracyclines					
TC	0	185			
OTC	0	1168	3974	107	662
CTC	0.5	213.7			
Amphenicols					
FF	1	10 000	56	10 000	56
Macrolides					
ETM	ND	2526	478	ND	ND

4. DISCUSSION

Sulfadiazine, sulfachloropyridazine and sulfaquinoxaline appeared to possess the greatest endocrine-disrupting potential due to their medium-high binding affinity score with AR. This may be due to their structural similarity to some common nonsteroidal anti-androgens, such as bicalutamide, enzalutamide and apalutamide (Crawford et al. 2018). They possess 2 benzene/benzene-like rings including pyridine, diazine, pyrimidine and pyrazine, which are linked by nitrogen and sulfur. It has been reported that the benzene-sulfonamide group might be associated with anti-androgenic activity (Roell & Baniahmad 2011). However, to our knowledge, no study has been conducted to investigate the anti-androgenic activity of sulfonamide antibiotics or their direct binding and interaction with AR. Overall, further experimental research is warranted to elucidate the potential impacts of antibiotics on endocrine systems, as well as confirming the binding and interaction between antibiotics and NHRs, especially for sulfonamide antibiotics.

Among the NHRs, the antagonistic conformation of AR was the most vulnerable conformation. Singh et al. (2013) demonstrated that norfloxacin could downregulate the expression of AR mRNA and induce testicular toxicity in male Japanese quails, supporting the potential anti-androgenic activity of the antibiotic ligands we found in the docking simulations. For GR, TRβ and TRα, 50 %, 36 % and 43 %, respectively, of individual bindings with the antibiotic ligands displayed medium probability, indicating potential endocrine-disrupting effects of aquaculture antibiotics mediated via them. Kwon et al. (2016) showed that the combination of sulfamethoxazole could exacerbate thyroid endocrine disorders induced by widely used bisphenol AF in adult male zebrafish, which was also consistent with our docking results. Our results indicate that the potential endocrine-disrupting activities of antibiotics used in aquaculture may be mainly mediated by AR *an*, GR and TRs. However, these *in silico* results require further examination and validation by additional experiments. Because Endocrine Disruptome does not consider any of the pharmacokinetic parameters, including bioaccumulation and metabolism, it makes a prediction only for the confirmation of interest. In addition, weak ligands with much higher binding constants would not be classified in the class with high binding probability but can still be problematic, especially in chronic exposure and for the ligands whose median effective concentration values were greater than 1 μM (Kolšek et al. 2014). In spite of these limitations with the reverse-docking method, given that very little information is available on the activities and mechanisms of endocrine disruption of antibiotics, rapid prediction of binding probabilities to a variety of NHRs would help experts make informed decisions on further testing, such as identifying potential antibiotics of high concern and susceptible conformations of NHRs. Furthermore, upon submission of the molecular SMILES to Endocrine Disruptome, pan-assay interfering compounds (PAINS) alerts (Devillers et al. 2015) can also be shown if PAINS are detected, which can help us filter out false-positives from the study (Plošnik et al. 2015).

Antibiotics have been put into large-scale use in order to reduce fish disease and increase fish production in aquaculture, and thus a large quantity of antibiotic residues has accumulated in fish (H. Wang et al. 2017, Chen et al. 2020). Increasing evidence suggests that antibiotics could lead to endocrine disorders, although they are not typical EDCs (Kim et al. 2017, Park & Kwak 2018, Yu et al. 2020). The binding affinity score results demonstrate that sulfonamide antibiotics may possess the greatest endocrine-disrupting potential. Moreover, the antagonistic conformation of AR was the most vulnerable conformation, followed by GR and TRs. In addition, the comprehensive scores of the food-borne health risks brought by the antibiotics vary greatly from country to country. The rank order of the health risk was fluoroquinolones > amphenicols > sulfonamides > β-lactams > tetracyclines in China, while it was β-lactams > tetracyclines > sulfonamides > amphenicols > fluoroquinolones in the USA. Since the relative molecular mass of macrolide is basically higher than 500, it was unable to bind NHRs due to its large structure. Its health risk may be the lowest. Therefore, the endocrine disruption risk for various categories of antibiotics used in aquaculture could be ranked for a specific country or region based on our developed risk-scoring and -ranking system, which could help prioritize large numbers of aquaculture antibiotics quickly and efficiently, thereby substantially decreasing costs and animal use for subsequent experimental research. It is worth mentioning that the withholding period is important in aquaculture. In the actual process of aquaculture, the residual level of antibiotics in the edible parts of aquatic products varies due to their different elimination half-lives, which determines the withholding period to a large extent. However, for a specific antibiotic, the elimination half-life varies greatly among different aquaculture species (Samuelsen 2006), temperature (Rairat et al. 2020a) and salinity (Rairat et al. 2020b). Therefore, the withholding period was not included in the risk-ranking system.

Based on our results, we propose that subsequent *in vitro* and *in vivo* experiments should focus on the evaluation of fluoroquinolone, amphenicol and sulfonamide antibiotics commonly used in aquaculture in China on the food-borne endocrine disruption risks to human health. It is worth noting that, in China, although the binding affinity scores for fluoroquinolone and amphenicol antibiotics were relatively low, as the most widely used and largest-consumed aquaculture antibiotics, their potential large number of residues posed the final higher risk levels. Nevertheless, given that most of the antibiotics displayed relatively low probability bindings with NHRs, they tend to bring generally low health risk. Importantly, further experimental research and investigation is warranted to examine and validate these *in silico* results. Furthermore, issues of spatial and temporal scale in delineating links between antibiotic usage in aquaculture and endocrine-disrupting risks from consumption of aquatic products should be addressed based on more detailed data collected in the future. In conclusion, the present study poses questions regarding public health and safety associated with endocrine-disrupting potentials from the consumption of antibiotic-containing fish and other animals. In addition, we provide a novel approach to determine antibiotics of high concern with potential endocrine-disrupting activities efficiently and effortlessly for regulation before experimental verification.

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