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Toxicologic effect and transcriptome analysis for short-term orally dosed enrofloxacin combined with two veterinary antimicrobials on rat liver☆

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ABSTRACT

Presently, toxicological assessment of multiple veterinary antimicrobials has not been performed on mammals. In this study, we assessed the short-term toxicity of enrofloxacin (E) combined with colistin (C) and quinocetone (Q). Young male rats were orally dosed drug mixtures and single drugs in 14 consecutive days, each at the dose of 20, 80, and 400 mg/(kg·BW) for environmental toxicologic study. The results showed that at the high dose treatment, the combination of E + C+Q significantly decreased body intake, lymphocytes count on rats; significantly increased the values of Alanine aminotransferase (ALT), Glutamic oxaloacetic transaminase (AST) and, cholinesterase (CHE); it also got the severest histopathological changes, where sinusoidal congestion and a large number of black particles in sinusoids were observed. This means E + C+Q in the high dose groups was able to cause significant damage to the liver. Other combinations or doses did not induce significant liver damage. Transcriptome analysis was then performed on rats in high dose group for further research. For E + C and E + Q, an amount of 375 and 480 differently expressed genes were filtered out, revealing their possible underlying effect on genomes. For E + C+Q, a weighted gene co-expression network analysis was performed and 96 hub genes were identified to reveal the specific effect induced by this combination. This study indicates that joint toxicity should be taken into consideration when involving the risk assessment of these antimicrobials.

1. Introduction

Recently, the risk of multiple chemicals, especially manmade chemicals, has become to public's awareness and concern (McEntaggart et al., 2019). Served as a large category of chemicals, veterinary antimicrobials are widely used for disease treatment, disease prevention, and growth promotion of animals (Grave et al., 2010, 2012; Van Boeckel et al., 2015). As a result, severe residues of antimicrobial mixtures may occur to animals. Some reports revealed that multiple veterinary drug contamination has also been existed in water, food and soil (Brown et al., 2020; Han et al., 2020). Thus, the assessment of the joint toxicity for veterinary drugs is of significance for ecological environment concerns and human health.

Enrofloxacin is a quinolone antibacterial drug dedicated to livestock and poultry and aquatic products for the broad-acting bacteriostatic effect that only used on animals (Babaahmady, 2011). Colistin (polymyxin E) has a strong antibacterial effect to gram-negative bacteria and is used for both animals and humans (Cai et al., 2015). The total global consumption of Colistin has reached 18 million standard units in 2010 and has a tendency to expand (Yen et al., 2021). Quinocetone

Abbreviations: E, enrofloxacin; C, colistin; Q, quinocetone; E + C, enrofloxacin + colistin; E + Q, enrofloxacin + quinocetone; E + C+Q, enrofloxacin + colistin + quinocetone; WBC, white blood cell; Neu, neutrocyte; Lym, lymphocyte; Mon, monocytes; Eos, Eosinophils; Bas, Basophils; RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; γ-GT, γ-glutamyl transferase; TP, total protein; CREA, creatinine; GLO, globulose; CHE, acetylcholinesterase; FPKM, Fragments Per Kilobase Millon Mapped Reads; DEGs, differentially expressed genes; GO, Gene Ontology; CC, cellular component; MF, molecular function; BP, biological process; KEGG, Kyoto Encyclopedia of Genes and Genomes; GS, gene significance; MS, module significance; qRT-PCR, quantitative reverse transcriptase-polymerase chain reaction.

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[1-(3-methyl-1,4-dioxide-2-quinoxalinyl)− 3-phenyl-2-propen-1-one] is an antimicrobial with rapid metabolism and low toxicity to animal (Chen et al., 2009). In 2018, its use has exceeded 1000 tons in China (Sun et al., 2019). Among all veterinary antimicrobials, these drugs would lead to a higher possibility for combined exposure due to their extensive use.

Toxicity data are the key to support the risk assessment of multiple chemicals (European 2019). There have been many reports on the toxicity of the above drugs based on single drug experiments. For instance, a study showed that enrofloxacin has immunosuppressive activity on chicks (Klaudia and Alina, 2015). Similarly, some clinical reports showed that colistin could induce nephrotoxicity and neurotoxicity (Falagas and Kasiakou, 2006). In vitro experiments indicated that quinocetone may have the potential to induce DNA damage, though no obvious toxicity was found in animal tests (Wang et al., 2010; Yang et al., 2018). However, not only the joint toxicity of the combination of these drugs, but of most other drugs, remains unknown.

In recent years, there were studies on the combined effect of veterinary drugs on model organisms. Tong Fei established a computational model to study the joint toxicity of tetracycline with copper (II) and cadmium (II) to Vibrio fischeri (Tong et al., 2015). Gomes studied the combined effects of amoxicillin, enrofloxacin, and oxytetracycline on Lemna minor, a possible indicator of service water pollution (Gomes et al., 2020). Wenhui Qiu found that early development and metabolic system of zebrafish larvae were affected while exposed to a mixture of four antibiotics (Qiu et al., 2020). All these studies observed an increase in toxicity while particular drugs were combined, and tended to arise more people's awareness on joint toxicity. Nevertheless, toxicity assessment of veterinary antimicrobial mixtures has not been studied on mammals, which are currently irreplaceable as toxicity data.

In this study, we selected young male Sprague-Dawley rats as a model animal for mammals (including humans) to analyze the toxic potential of enrofloxacin, polymyxin E sulfate, quinocetone, and their combination. This study examined the liver damage after a 14-days oral dosing of each single drugs and their mixtures. Then, high-throughput transcriptome sequencing (RNA-seq) was used to seek for possible mechanisms of combined toxic of the drug mixtures.

2. Methods

2.1. Chemical and reagents

Enrofloxacin (>98%), colistin (>19000 U/mg), and quinocetone (>95%) were purchased from Hubei Weideli Chemical Technology Co., Ltd. (Hubei, China).

2.2. Animals

SPF Sprague-Dawley male rats were 3 – 4 weeks old and weighted 65–75 g, purchased from SPF Biotechnology Co., Ltd. (Beijing, China). After rats with poor physical condition were removed, a total of 105 male rats were used for the toxic study. All rats were housed at 18–25 ℃, 30–60% humidity, and exposed to 12 h of daily light. The rats were fed normal rat maintenance diet and drank distilled water. They were acclimated to the experimental environment for 5 days prior to the experiment. All procedures followed the guidance of the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals and approved by the Animal Welfare and Animal Experimental Ethical Committee at the China Agricultural University (certification no: AW32010202–2–2).

2.3. Experimental design

105 male rats were randomly divided into seven experimental groups [control, enrofloxacin(E), colistin(C), quinocetone(Q), enrofloxacin + colistin(E + C), enrofloxacin + quinocetone(E + Q), enrofloxacin + colistin + quinocetone(E + C+Q)], with 15 rats in each group. In each group, the administrated dose was 20, 80, and 400 mg/(kg·BW) (for each drug in the joint treatment groups) at three concentrations, 5 rats per concentration. Drugs were dissolved in 0.5% sodium carboxymethylcellulose water solution, and 0.4 ml drug solution was administered per 100 g body weight each day for 14 consecutive days. At 24 h after the last day of dosing, the rats were anesthetized by ether. For each rat, heart blood was collected into an anticoagulant tube for hematological analysis and a non-anticoagulant tube for biochemical analysis. Then the rats were anesthetized with chloral hydrate and sacrificed by cervical dislocation. Finally, liver tissues were collected and stored at −80 ℃ immediately for subsequent testing.

2.4. Hematology and biochemical analysis

Hematology analysis was performed on Mindray BC5000 Hematology Analyzer (Mindray, Shenzhen, China). Blood samples from non-anticoagulant tubes were centrifuged at 5000 rpm for 3 min to obtain serum. Serum was loaded on Mindray BS-240 Biochemistry Analyzer (Mindray, Shenzhen, China). The following parameters were measured: white blood cell (WBC), neutrocyte (Neu), lymphocyte (Lym), monocytes (Mon), Eosinophils (Eos), Basophils (Bas), red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyl transferase(γ-GT), total protein (TP), creatinine (CREA), globulose (GLO), and acetylcholinesterase (CHE).

2.5. Histopathological examination

A portion of the liver tissue was stored in 10% buffered formalin after collection. After dehydration, immersing, and paraffin embedding, 2–3 μm sections were cut and stained with hematoxylin-eosin (HE). Tissue sections were examined microscopically at 200 × or 400 × magnification.

2.6. RNA extraction

HiPure Universal RNA Mini Kit (Magen, Guangzhou, China) was used to extract total RNA from liver tissue. The purity of RNA was checked using Nanodrop2000 spectrophotometer (Thermo fisher, MA, USA). RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA) was used to determine the integrity of RNA samples. Qualified RNA samples were also used for quantitative PCR analysis.

2.7. Library construction, transcriptome sequencing, and genome alignment

Library Prep Kit for Illumina® #E7530L (NEB, USA) was used to generate sequencing libraries following the manufacturer's recommendations. HiSeq PE Cluster Kit V4 cBot (Illumina, CA, USA) was used for library clustering. The libraries were then sequenced on Illumina NovaSeq 6000 (Illumina, CA,http://www.kegg.jp/ USA) for 3 GB raw data. Cutadapt v2.10 was performed to remove adapter sequence primers, poly-A tails and sequences of lower quality from raw sequence reads (Martin 2011). HISAT v2.2.1 along with indexes for Rattus norvegicus (downloaded from https://daehwankimlab.github.io/hisat2/download/) was used for genome alignment (Kim et al., 2015).

2.8. Bioinformatics analyses of the sequencing results

Subread v2.0.1 was used to count the reads count for each gene in each sample (Liao et al., 2019). The expression level of genes in each sample was calculated as a standardized quantitative representation, the

Table 1
Body weight of Sprague Dawley rats in each treatment.

Group	Day 0			Day 14			Day 14 - Day 0		
	400 mg/(kg·BW)	80 mg/(kg·BW)	20 mg/(kg·BW)	400 mg/(kg·BW)	80 mg/(kg·BW)	20 mg/(kg·BW)	400 mg/(kg·BW)	80 mg/(kg·BW)	20 mg/(kg·BW)
Ctrl	87.2 ± 2.8			188.9 ± 10.3			101.7 ± 8.0		
E	87.8 ± 1.9	87.3 ± 4.1	87.3 ± 4.7	187.1 ± 9.7	185.1 ± 8.1	187.3 ± 5.4	99.3 ± 8.3	97.7 ± 8.7	100.1 ± 3.3
P	91.8 ± 2.9	88.0 ± 8.0	90.0 ± 4.2	208.6 ± 9.1*	186.0 ± 19.3	197.6 ± 13.9	116.8 ± 7.7*	98.1 ± 12.9	108.7 ± 13.1
Q	85.7 ± 2.0	87.7 ± 2.6	87.4 ± 5.5	186.5 ± 5.2	185.5 ± 16.8	198.2 ± 25.9	100.8 ± 5.6	97.7 ± 14.9	109.8 ± 23.1
E + P	87.3 ± 1.9	90.8 ± 4.2	88.7 ± 3.8	175.1 ± 13.5	188.2 ± 8.4	193.3 ± 7.9	87.8 ± 12.5	97.4 ± 6.5	102.6 ± 6.0
E + Q	87.8 ± 5.0	91.2 ± 4.0	86.8 ± 2.5	172.7 ± 7.2*	193.6 ± 17.1	188.9 ± 4.6	84.9 ± 4.4*	102.4 ± 14.5	102.1 ± 5.2
E + P + Q	92.9 ± 3.3	88.5 ± 3.5	89.8 ± 5.8	166.6 ± 14.2	184.9 ± 12.1	185.2 ± 16.1	73.7 ± 11.9*	96.4 ± 9.9	96.3 ± 11.5

Results are expressed as mean ± SD.
* represents significantly different from the control group ($p < 0.05$).

FPKM (Fragments Per Kilobase Millon Mapped Reads). Differences in gene expression between the control group and multiple drug treatment group were analyzed using R package Deseq2. DESeq2 estimates the expression level of each gene per sample by the linear regression, then calculates a p-value, which was afterward adjusted by the Benjamini's approach as q value. Genes with $q \leq 0.05$ and $|log2\_ratio| \geq 1$ are identified as differentially expressed genes (DEGs). The selected differential genes were GO and KEGG enriched using an R package ClusterProfiler. GO, known as Gene Ontology (http://geneontology.org/), is a database that divides the function of genes into three parts: (cellular component, CC), (molecular function, MF), and (biological process, BP). KEGG (Kyoto Encyclopedia of Genes and Genomes, http://www.kegg.jp/), is a database associated with genome deciphering. KEGG and GO terms with $q < 0.05$ were considered to be significantly enriched.

Weighted gene co-expression network analysis (WGCNA) was used to identify the specific differentially expressed genes induced by the ternary drug combination (Langfelder and Horvath, 2008). Instead of relating all the genes to sample groups, it divides all genes into several modules (a cluster of highly interconnected genes) and focuses on the relationship between modules and the sample groups. The correlation between genes and sample groups was calculated as Gene significance (GS). The average GS for all genes in a given module was calculated as module significance (MS). In this study genes with $GS > 0.2$ and $MS > 0.8$ were recognized as hub genes that responsible for joint toxicity for ternary drug combination.

2.9. Validation of quantitative PCR analysis

The expression levels of DEGs and hub genes in the rats were validated using quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR). cDNA was generated from 1 μg total RNA of each sample using PrimeScript RT Master Mix (Takara, MA, USA). PCR was performed using TB Green™ Premix Ex Taq™ according to the manufacturer's instruction. Then PCR was processed on 7500 Real-Time PCR System (Thermo Fisher Scientific) in a total reaction volume of 20 μl. Primer sequences were shown in Table S1. The thermal cycling conditions were as follow: 95 °C for 3 min, 40 cycles at 95 °C for 5 s, and 60 °C for 34 s. For quantitative results, the relative expression level of each genome was calculated using the $2^{-\Delta\Delta Ct}$ method.

2.10. Statistical analysis

Bodyweight, hematology, and biochemical analysis results were expressed as mean ± standard deviation ($n = 5$). One-way analysis of variance (ANOVA) and Tukey's test were used to compare the means of multiple groups if they passed Levene's Test. P-values < 0.05 were considered to be statistically significant. The python v3.9 package NumPy (v1,1,9) and SciPy (v1.6.0) were used to carry out all statistical analyses.

3. Results and analysis

3.1. Bodyweight

As shown in Table 1, at the dose of 400 mg/(kg·BW), compared with the control group, the bodyweight of the combination of E + Q and E + C+Q were markedly decreased, while single C showed an opposite change. All treatments at the dose of 80 mg/(kg·BW) and 20 mg/(kg·BW) had no obvious influence on the body weight. These results suggest that the combination of E + Q and E + C+Q may have joint toxicity.

3.2. Hematology and biochemical analysis

Hematology analysis results were shown in Table 2. Oral administration of antimicrobials in single and combination had little effect on the hematology analysis results of rats. However, at the dose of 400 mg/(kg·BW), rats in group C significantly reduced MCV, MCH, and MCHC compared to the control group; while that in group E + C significantly decreased MCH. In addition, group E + C+Q significantly decreased lymphocyte count in rats. Results also indicate E + Q and E + C+Q may have joint toxicity.

Several biochemical parameters related to liver function were measured. Results were shown in Table 3. Biochemical parameters changed significantly for group C, E + Q, and E + C+Q. For group C, the value of ALB and TP was significantly decreased at 400 mg/(kg·BW) compared to the control group, the value of GLO was reduced at all doses, and the value of CHE was decreased at 80 mg/(kg·BW) as well as 20 mg/(kg·BW). For Group E + Q, the value of ALP was significantly decreased at all doses, while the value of γ-GT was markedly reduced at 80 mg/(kg·BW) and 20 mg/(kg·BW) compared to the control group. For group E + C+Q, however, the value of ALT, AST and, CHE significantly increased at 400 mg/(kg·BW). Besides, a significantly decline was observed in the value of ALP at all doses compared to the control group. These results mean that joint toxicity may lead to these changes.

3.3. Histopathological analysis

To verify the possible toxicity of single drug and drug combinations, histopathological studies were performed. No significant changes were observed between the control group and the rats in the 80 mg/(kg·BW) and 20 mg/(kg·BW) treatment groups (data not shown). In this study, the liver section of the control group and all treatment at 400 mg/(kg·BW) were shown in Fig. 1. Liver tissues in the control group were normal, while slight swelling of hepatocytes was observed around the central vein in group E and group E + C. Dissociation of hepatic cord has been observed in group C. In group Q, there was inflammatory cell infiltration in portal areas. No obvious pathological lesions were found in group E + Q, indicating that the liver may not be the main site if this

Table 2
Hematology analysis result of Sprague Dawley rats after antimicrobials orally treated in single and combined.

Group	WBC ($\times10^9$/L)			Neu ($\times10^9$/L)			Lym ($\times10^9$/L)			Mon ($\times10^9$/L)		
	400 mg/(kg·BW)	80 mg/(kg·BW)	20 mg/ (kg·BW)	400 mg/(kg·BW)	80 mg/(kg·BW)	20 mg/(kg·BW)	400 mg/(kg·BW)	80 mg/(kg·BW)	20 mg/(kg·BW)	400 mg/(kg·BW)	80 mg/(kg·BW)	20 mg/ (kg·BW)
Ctrl	6.44 ± 1.05			0.62 ± 0.22			5.42 ± 0.95			0.31 ± 0.12		
E			6.34 ± 1.62									0.36 ± 0.19
	5.25 ± 2.30	6.31 ± 0.66		0.69 ± 0.39	0.6 ± 0.11	0.65 ± 0.27	4.26 ± 1.94	5.3 ± 0.52	5.21 ± 1.21	0.16 ± 0.05	0.32 ± 0.05	
P			6.53 ± 1.87									0.34 ± 0.11
	8.10 ± 3.00	7.34 ± 2.01		0.65 ± 0.22	0.96 ± 0.47	0.73 ± 0.24	6.86 ± 2.44	5.71 ± 1.55	5.30 ± 1.73	0.46 ± 0.28	0.48 ± 0.27	
Q			5.64 ± 1.89									0.24 ± 0.07
	6.41 ± 3.50	6.26 ± 2.42		0.95 ± 0.56	0.94 ± 0.22	0.72 ± 0.22	5.12 ± 2.79	4.8 ± 2.04	4.56 ± 1.68	0.18 ± 0.05	0.42 ± 0.25	
E + P			6.38 ± 1.07									0.32 ± 0.18
	5.55 ± 2.28	6.15 ± 1.4		1.13 ± 0.68	0.55 ± 0.19	0.81 ± 0.22	4.01 ± 1.66	5.09 ± 0.77	5.09 ± 0.77	0.31 ± 0.19	0.40 ± 0.22	
E + Q			9.09 ± 5.79									0.44 ± 0.33
	7.21 ± 2.11	6.55 ± 2.07		1.07 ± 0.41	0.61 ± 0.14	1.44 ± 1.25	5.60 ± 1.70	5.44 ± 1.93	7.01 ± 4.16	0.42 ± 0.12	0.37 ± 0.09	
E + P + Q			5.91 ± 1.17									0.33 ± 0.2
	4.29 ± 1.78	5.24 ± 1.14		0.98 ± 0.98	0.61 ± 0.25	0.81 ± 0.35	**2.93 ± 1.03***	**4.24 ± 1.04***	4.68 ± 0.75	0.25 ± 0.09	0.28 ± 0.06	

Group	Eos ($\times10^9$/L)			Bas ($\times10^9$/L)			RBC ($\times10^{12}$/L)			HGB (g/L)		
	400 mg/(kg·BW)	80 mg/(kg·BW)	20 mg/(kg·BW)	400 mg/(kg·BW)	80 mg/(kg·BW)	20 mg/(kg·BW)	400 mg/(kg·BW)	80 mg/(kg·BW)	20 mg/(kg·BW)	400 mg/(kg·BW)	80 mg/(kg·BW)	20 mg/(kg·BW)
Ctrl	0.03 ± 0.01			0.06 ± 0.02			6.92 ± 0.74			149 ± 16		
E			0.06 ± 0.05									151 ± 16
	0.04 ± 0.02	0.04 ± 0.01		0.09 ± 0.05	0.06 ± 0.03	0.06 ± 0.04	6.31 ± 0.71	6.51 ± 0.61	7.06 ± 0.56	137 ± 15	143 ± 14	
P			0.07 ± 0.04									165 ± 22
	0.07 ± 0.06	0.08 ± 0.04		0.07 ± 0.05	0.10 ± 0.02	0.09 ± 0.03	8.18 ± 0.95	6.71 ± 0.8	7.30 ± 1.05	163 ± 19	146 ± 20	
Q			0.05 ± 0.03									157 ± 18
	0.02 ± 0.02	0.05 ± 0.02		0.13 ± 0.12	0.08 ± 0.02	0.06 ± 0.02	6.94 ± 0.22	7.43 ± 0.93	7.16 ± 0.79	146 ± 6	160 ± 16	
E + P			0.06 ± 0.04									150 ± 7
	0.05 ± 0.03	0.06 ± 0.02		0.05 ± 0.06	0.06 ± 0.02	0.09 ± 0.05	6.78 ± 1.47	7.57 ± 1.25	6.88 ± 0.25	139 ± 29	167 ± 28	
E + Q			0.11 ± 0.12									161 ± 177
	0.05 ± 0.02	0.04 ± 0.02		0.08 ± 0.05	0.09 ± 0.03	0.09 ± 0.07	7.16 ± 1.10	7.27 ± 1.3	7.42 ± 0.59	154 ± 22	153 ± 25	
E + P + Q			0.04 ± 0.02									128 ± 24
	0.09 ± 0.09	0.03 ± 0.01		0.03 ± 0.02	0.07 ± 0.02	0.05 ± 0.04	7.03 ± 0.80	6.77 ± 0.29	6.00 ± 1.23	146 ± 24	144 ± 6	

Group	MCV (fL)			MCH (pg)			MCHC (g/L)			PLT (10^3/L)		
	400 mg/(kg·BW)	80 mg/(kg·BW)	20 mg/(kg·BW)	400 mg/(kg·BW)	80 mg/(kg·BW)	20 mg/(kg·BW)	400 mg/(kg·BW)	80 mg/(kg·BW)	20 mg/(kg·BW)	400 mg/(kg·BW)	80 mg/(kg·BW)	20 mg/(kg·BW)
Ctrl	66.3 ± 1.9			21.5 ± 0.3			325 ± 7			672 ± 243		
E	65.2 ± 2.5									742 ± 101		560 ± 258
		67.0 ± 0.7	65.3 ± 3.2	21.7 ± 0.8	22.0 ± 0.2	21.4 ± 1.1	333 ± 8	328 ± 7	328 ± 7	765 ± 478		
P	**63.4 ± 0.8***									422 ± 319		536 ± 271
		65.0 ± 1.3	67.8 ± 2.8	**20.0 ± 0.2***	21.7 ± 0.5	**22.6 ± 1.6***	**315 ± 3***	**335 ± 6***	**334 ± 13***	832 ± 291		
Q	64.1 ± 1.5									689 ± 225		529 ± 382
		65.8 ± 2.7	65.5 ± 3.3	21.0 ± 0.4	22.0 ± 1.5	21.9 ± 0.8	328 ± 5	334 ± 16	334 ± 7	905 ± 430		
E + P	63.3 ± 2.6									535 ± 422		890 ± 271
		66.0 ± 1.6	65.5 ± 1.7	**20.5 ± 0.8***	22.0 ± 0.6	21.7 ± 0.5	325 ± 9	333 ± 5	332 ± 8	1125 ± 671		
E + Q	65.3 ± 1.9									724 ± 314		790 ± 490
		64.1 ± 1.2	66.5 ± 2.5	21.5 ± 0.6	21.1 ± 0.6	21.7 ± 0.8	329 ± 4	330 ± 7	326 ± 5	595 ± 351		
E + P + Q	63.2 ± 3.8									579 ± 306		729 ± 181
		65.5 ± 0.8	64.9 ± 3.6	20.7 ± 1.3	21.3 ± 0.3	21.0 ± 1.3	328 ± 2	326 ± 4	326 ± 8	720 ± 580		

Results are expressed as mean ± SD.
* represents significantly different from the control group ($p < 0.05$).

Table 3
Biochemical analysis result of Sprague Dawley rats after antimicrobials orally treated in single and combined.

Group	ALB/ (g·L-1)			ALP/ (U·L-1)			ALT/ (U·L-1)			AST/ (U·L-1)		
	400 mg/ (kg·BW)	80 mg/ (kg·BW)	20 mg/ (kg·BW)	400 mg/ (kg·BW)	80 mg/ (kg·BW)	20 mg/ (kg·BW)	400 mg/ (kg·BW)	80 mg/ (kg·BW)	20 mg/ (kg·BW)	400 mg/ (kg·BW)	80 mg/ (kg·BW)	20 mg/ (kg·BW)
Ctrl	33.2 ± 0.9			193.4 ± 17.6			68.2 ± 10.5			153.2 ± 16.7		
E	33.6 ± 1.5	32.2 ± 1.2	33.1 ± 1.4	181.1 ± 30.7	135.0 ± 57.8	153.4 ± 62.2	64.1 ± 15.0	72.9 ± 10.5	149.0 ± 202.0	169.8 ± 37.2	165.8 ± 49.0	392.3 ± 449.2
P	31.3 ± 1.8*	33.3 ± 0.7	33.5 ± 0.6	203.2 ± 20.3	137.6 ± 80.1	146.8 ± 31.7	65.3 ± 9.2	82.8 ± 12.0	62.9 ± 15.3	133.3 ± 21.0	221.0 ± 86.9	168.1 ± 59.2
Q	33.5 ± 2.1	31.5 ± 1.1	32.1 ± 1.4	161.8 ± 34.7	171.6 ± 52.3	167.0 ± 37.7	46.7 ± 7.0*	60.6 ± 11.6	59.0 ± 12.1	147.3 ± 18.6	128.8 ± 28.8	128.7 ± 3.8
E + P	34.3 ± 1.9	32.7 ± 1.0	32.3 ± 1.2	189.5 ± 43.2	164.0 ± 80.7	170.7 ± 65.5	64.0 ± 11.3	68.8 ± 10.0	55.5 ± 8.0	154.2 ± 37.7	131.8 ± 17.3	121.3 ± 18.5
E + Q	33.3 ± 1.7	32.9 ± 1.1	27.5 ± 13.0	106.7 ± 37.1*	137.4 ± 57.0*	138.3 ± 28.8*	59.8 ± 12.6	56.5 ± 13.7	57.6 ± 11.3	188.7 ± 53.5	128.3 ± 12.4	152.3 ± 46.8
E + P + Q	32.0 ± 0.5	31.9 ± 0.8	32.6 ± 2.4	104.4 ± 28.5*	151.1 ± 37.0*	158.5 ± 20.7*	84.5 ± 7.7*	78.0 ± 13.0	60.2 ± 18.0	294.9 ± 132.5*	134.8 ± 11.1	141.2 ± 20.8

Group	CHE/ (U·L-1)			Glo (g/·L-1)			TP (g·L-1)			γ-GT (U·L-1)		
	400 mg/ (kg·BW)	80 mg/ (kg·BW)	20 mg/ (kg·BW)	400 mg/ (kg·BW)	80 mg/ (kg·BW)	20 mg/ (kg·BW)	400 mg/ (kg·BW)	80 mg/ (kg·BW)	20 mg/ (kg·BW)	400 mg/(kg·BW)	80 mg/ (kg·BW)	20 mg/ (kg·BW)
Ctrl	311.0 ± 47.0			25.1 ± 2.0			58.3 ± 2.8			1.1 ± 0.5		
E	249.0 ± 82.0	270.8 ± 49.5	322.0 ± 37.0	25.3 ± 3.9	22.8 ± 3.0	24.9 ± 1.2	58.9 ± 5.3	54.0 ± 3.9	58.0 ± 1.8	1.1 ± 0.9	1.6 ± 0.9	1.6 ± 0.3
P	321.0 ± 66.0	225.4 ± 11.7*	259.0 ± 51.0*	23.2 ± 0.7*	22.5 ± 1.5*	25.3 ± 1.4	52.8 ± 2.5*	56.5 ± 0.9	58.7 ± 1.6	1.6 ± 0.5	1.9 ± 0.3	1.4 ± 0.6
Q	346.0 ± 21.0	288.0 ± 44.6	271.0 ± 42.0	23.7 ± 2.6	20.9 ± 5.2	24.3 ± 2.2	57.2 ± 4.7	52.3 ± 5.4	56.5 ± 3.2	1.6 ± 0.6	1.8 ± 0.7	1.7 ± 0.3
E + P	346.0 ± 215.0	295.8 ± 41.3	282.0 ± 44.0	22.6 ± 3.0	24.0 ± 1.2	23.0 ± 2.0	57.0 ± 4.2	55.7 ± 1.4	54.2 ± 1.4	3.0 ± 1.4*	1.9 ± 0.7	1.7 ± 0.4
E + Q	314.0 ± 75.0	300.0 ± 63.7	340.0 ± 36.0	22.8 ± 2.7	23.4 ± 2.0	29.6 ± 12.6	56.1 ± 4.1	56.2 ± 2.9	57.1 ± 1.2	1.1 ± 0.7	1.9 ± 0.4 *	**2.1 ± 0.4** *
E + P + Q	455.0 ± 70.0*	300.8 ± 61.3	322.0 ± 49.0	21.6 ± 1.7	23.5 ± 1.7	21.9 ± 2.2	53.6 ± 1.9	55.4 ± 1.6	54.5 ± 4.5	2.0 ± 0.7	1.4 ± 0.3	1.6 ± 0.4

Results are expressed as mean ± SD.
* represents significantly different from the control group ($p < 0.05$).

combination produces joint toxicity. The severest histopathological changes were found in group E + C+Q, where sinusoidal congestion and a large number of black particles (may be drugs unmetabolized) in sinusoids were observed.

3.4. Identification of differently expressed genes

To identify minor changes caused by drug mixtures, the total RNA of liver tissues in all treatments at 400 mg/(kg·BW) were extracted and used for RNA-seq. Three biological replicates in each treatment (21 samples in all) performed RNA-seq. DEGs were obtained after comparing the FPKM values of all detected genes in each treatment with the control group. As shown in Fig. 2A, the total DEGs for group E, group C, group Q, group E + C, group E + Q, group E + C+Q were 208, 143, 335, 375, 480 and, 585, respectively. In this study, we mainly focused on gene changes induced by multiple antimicrobials. Overlapping genes between two binary drug combinations and their compositions were presented as a Venn diagram in Fig. 2B and C. Relative expression of some DEGs was presented in Fig. 2D.

To better identify the genes induced by multiple antimicrobials treatment, GO and KEGG analyzes were used to evaluate the DEGs. As shown in Fig. 3A and B, for group E + C, several biological processes such as chromosome localization (a process where chromosome is transported to or maintained in a specific location), metaphase plate congression (the alignment of chromosomes at the metaphase plate), and glycogen metabolic process were enriched in the GO database. Biological pathways such as cell cycle, cysteine, and methionine metabolism (vital for amino acid metabolism), p53 signaling pathway (related with cell cycle arresting) were enriched in the KEGG database. In all, for group E + C, DEGs were mainly related with cell cycle and metabolic process. For group E + Q, however, the DEGs mainly enriched in metabolic related process after both GO and KEGG analysis (see Fig. 3C and D).

3.5. WGCNA for identification of hub genes responsible for ternary drug treatment

For ternary combination, it's hard to distinguish the combined effect of ternary combination from its component through one-by-one comparison. Thus, in this study, we used WGCNA to identify the effect of

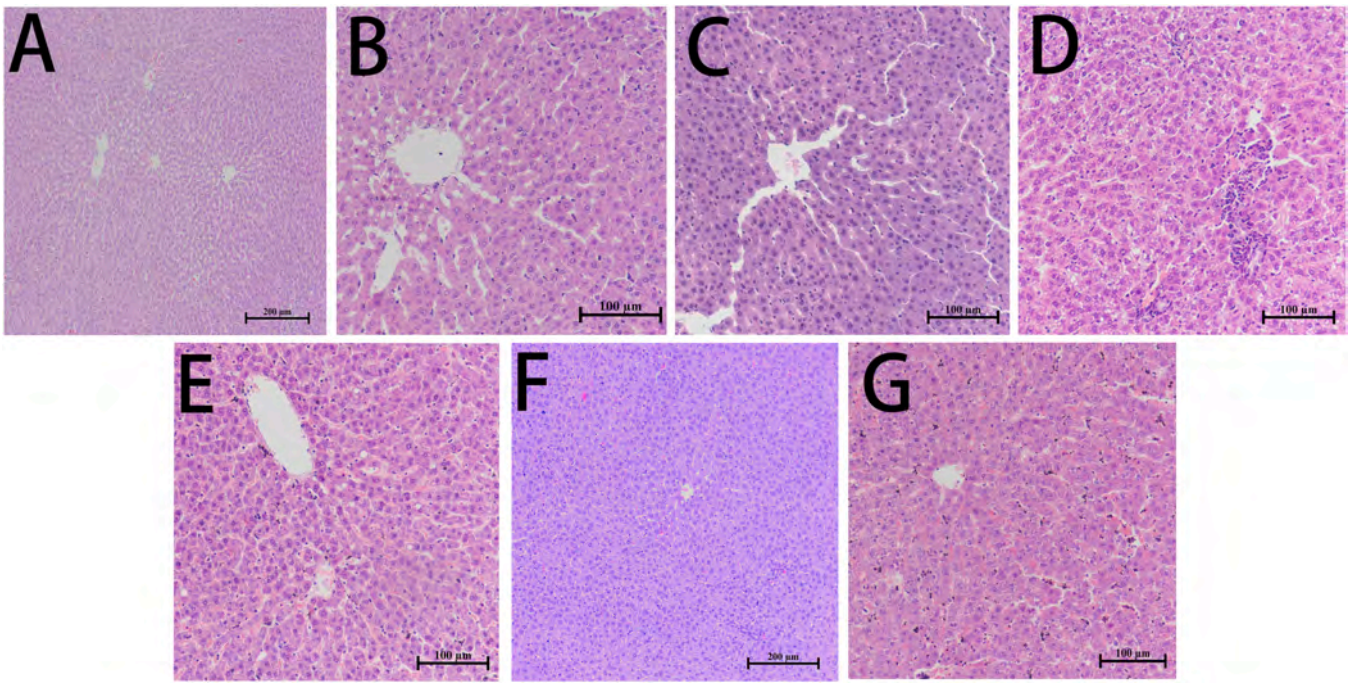

Fig. 1. Histopathological results of liver tissues in rats after antimicrobials orally treated in single and combined. A(control, 200 ×); B(enrofloxacin, 400 ×); C (colistin, 400 ×); D(quinocetone, 400 ×); E(enrofloxacin + colistin, 400 ×); F(enrofloxacin + quinocetone, 200 ×); G(enrofloxacin + collistin + quinocetone, 400 ×).

joint toxicity on genomes. Top 5000 genes that differ between different samples were partitioned into multiple modules based on a specific statistical distance between them. It can be seen in Fig. 4A and D that these genes were divided into 28 modules. Of all the modules, module MEtuoquise had the highest correlation with ternary drug treatment, with a Pearson Correlation of 0.87. There were 96 genes defined as hub genes after being selected using a GS and MS cutoff, which were then GO and KEGG analyzed (Fig. 4B and C). In GO analysis, these genes were mainly enriched in immune cell proliferation, metabolic process, and MHC-class II related process. In KEGG analysis, hub genes were also enriched in biological functions related with MHC-class II molecules and metabolic process.

3.6. Validation of DEGs and hub genes using qRT-PCR

There were five random DEGs and eight random hub genes selected for qRT-PCR validation. As shown in Fig. 2D and Fig. S1, relative expression five DEGs Cyp3a9, Cenpq, Ccnb1, Fam83d, and cenpf were significantly downregulated in group E + C and E + Q compared with the control group. Likewise, for eight hub genes, the relative expression of Cyp2c11, Cyp2a2, Cyp2c13, and Gsta2 were significantly downregulated in group E + C+Q compared with all other groups, while the relative expression of Cyp2c12, RT1-Db1, RT1-Bb, and Abcc2 were significantly upregulated. This indicates that the selected hub genes were specifically changed after ternary drug treatment of E + C+Q. All qRT-PCR results were relatively coincident with RNA-seq results.

4. Discussion

Besides clinical uses, veterinary antimicrobials are also added to the diet as growth-promoting agents. At present, however, there were few instructions of the combined use of veterinary drugs. Short-term use of these agents in animals can happen at a relatively high quantity in combination. One of the drugs we selected, enrofloxacin, was reported to be a cytochrome P450 inhibitor and was considered to have the potential to interact with other drugs (Li et al., 2018; Vancutsem and Babish, 1996). In this study, we performed a short-term and high-dose toxicologic study for enrofloxacin combined with another two antimicrobials. The high, medium and low concentrations used in this study were equivalent to 20 times, 4 times, and 1 time of the normal clinical dosage of each drug for animals, respectively. Since liver is the target organ for all drugs, this study mainly focuses on liver damage. Large amounts of antibiotic mixtures in animals are not completely absorbed but are released with manure and remain in the environment (Huygens et al., 2021) Thus, for ecotoxicological concerns, this study can also give fundamental data for the prediction of long-term and low-dose toxicity. Moreover, humans can expose to multiple veterinary antimicrobials by the ingestion of animal-derived foods. The MRL (maximum residue limits) can be a reference of the possible exposed concentration. In China and EU, the MRLs for enrofloxacin and colistin were 100 μg/kg and 150 μg/kg (in chicken muscle) respectively (Ministry of Agriculture et al., 2019; Eu commission, 2013).

Rats and mice are the most commonly used animal models in human toxicology experiments. Among them, Sprague Dawley rat is the specified animal species for drug toxicology evaluation in new drug approval applications in various countries. What's more, the acute toxicity, subchronic toxicity, and chronic toxicity data of single compounds in many public toxicity databases use Sprague Dawley rats as animal models. Therefore, we selected it as the animal model to study the possible combined toxic effects of mixed pollution of multiple veterinary drugs in animals.

In this study, no obvious hematology, blood biochemistry, and pathological changes were found at the dose of 20 mg/(kg·BW) for each drug combination, which suggested that short-term combined use of these drugs at clinical doses could not produce significant damage to the liver. However, minor changes induced by the combined effect may not be detected by regular biomarkers. To seek for the potential combined effect, we performed an RNA-seq on the liver tissues of rats in the high dose group. Consequently, a series of genes were found to be regulated by the combined use of antimicrobials.

Bodyweight is an intuitive indicator for toxicological studies. The significant decrease of body weight in group E + Q and E + C+Q

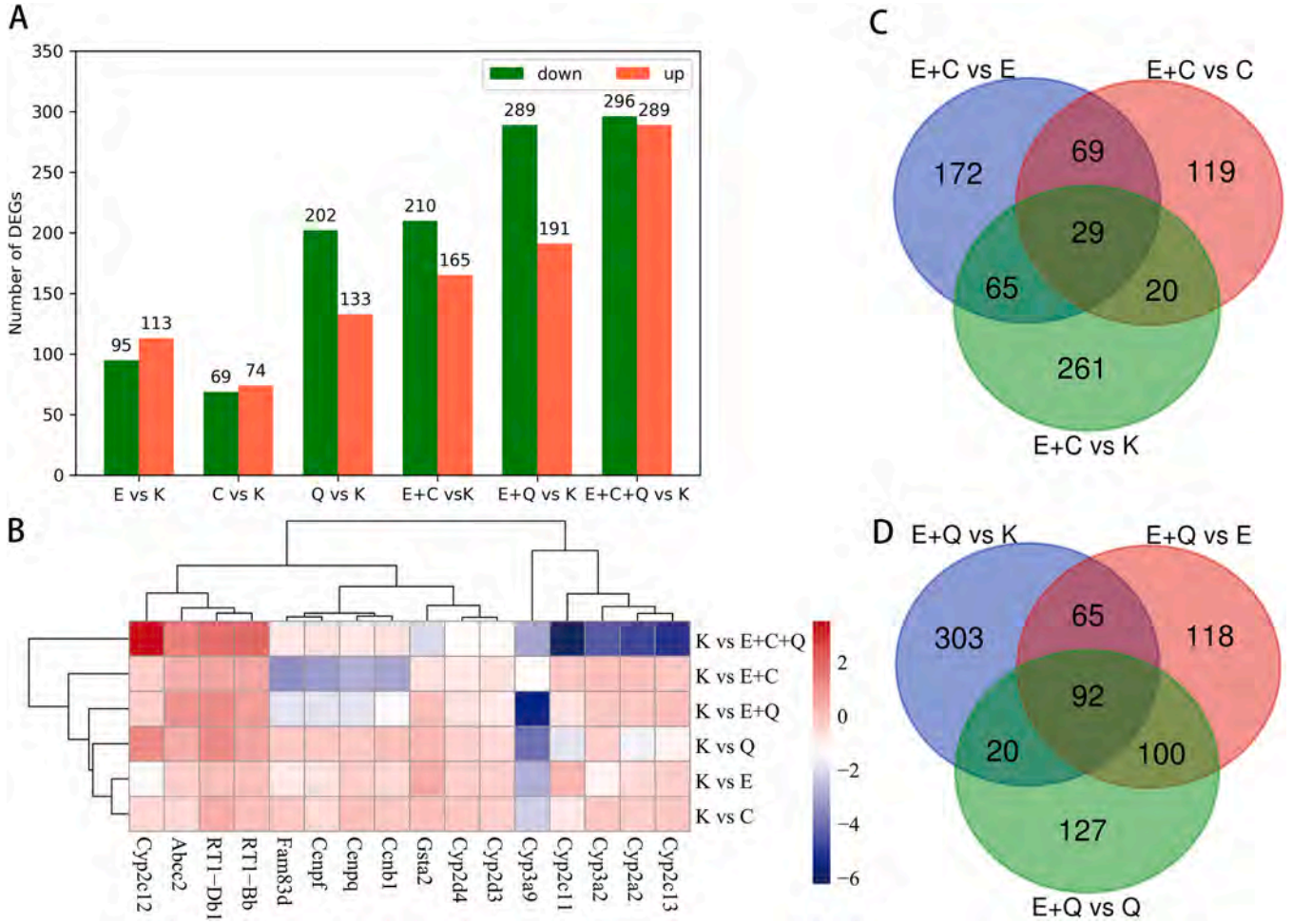

Fig. 2. (A) The number of differentially expressed genes (DEGs) in different drug treatments compared to that of the control group. (B) Hierarchical cluster analysis of 16 common genes and their relative expression in each drug treatment compared to that of the control group. (C) Venn diagram of group E + C and its components. (D) Venn diagram of group E + Q and its components.

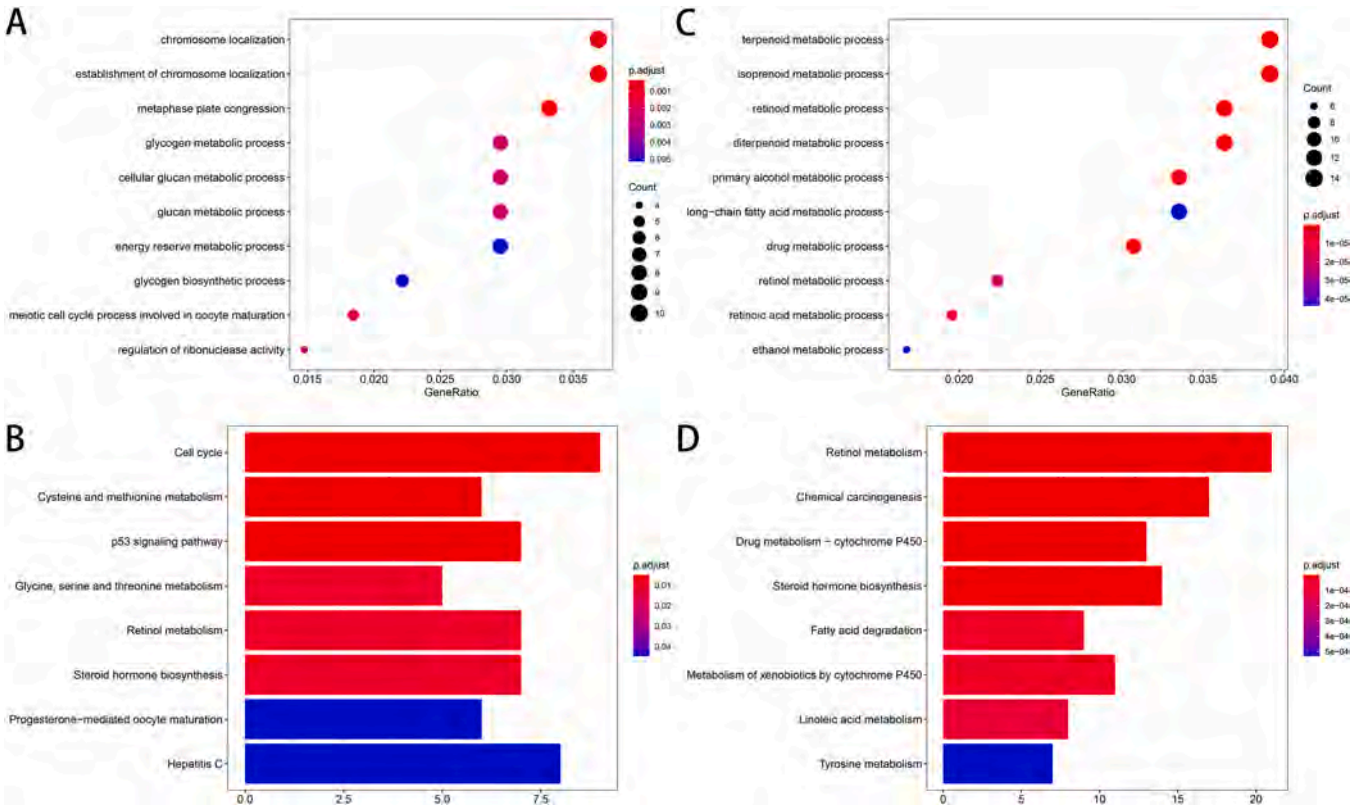

Fig. 3. (A) GO analysis results (biological process) of DEGs between the control group and group E + C. (B) KEGG analysis results of DEGs between the control group and group E + C. (C) GO analysis results (biological process) of DEGs between the control group and group E + Q. (D) KEGG analysis results of DEGs between the control group and group E + Q.

suggests that a high overall toxicity was possibly caused at the dose of 400 mg/(kg·BW). Lymphocytes, including T cells, B cells, and NK cells participate in immune regulation and antibody production. Thus, lymphocyte count represents the body's anti-infective ability (Espersen et al., 1995). Enrofloxacin was reported to have the ability to suppress T cells and B cells in chick but not in rats (Klaudia and Alina, 2015). In this study, however, the immunosuppressive potential of enrofloxacin was rediscovered while combined with colistin and quinocetone. ALP is a parameter possibly responsible for liver damage (Ozer et al., 2008). ALT and AST are reported to be sensitive parameters for liver disfunction (Li et al., 2020; Yuan et al., 2014). In group E + C+Q, the significant changes of ALP, ALT, and AST at the dose of 400 mg/(kg·BW) suggested that the combined use of these drugs may induce further hepatoxicity. Besides, the significant increase of CHE in this group indicated a low-grade systemic inflammation as well (Das, 2007). The combined use of enrofloxacin and quinocetone could also induce slight liver damage as γ-GT, which is also regarded as a liver function indicator, had a mild increase compared with the control group (Jia et al., 2014). Histopathological analysis confirmed the E + C+Q induced liver damage. While sinusoidal congestion indicated severe disruption of liver function, impurity stasis in sinusoids is a sign for hepatic metabolic capacity reduction.

Transcriptome analysis is a useful tool for uncovering regulatory pathways. In this study, the amount of DEGs in combined treatment groups was larger than that in single drug treatment groups while compared with the control group. The DEGs of group E + C was enriched in several cell cycle related pathways at G2/M stage, among which, Cenpf was reported to be associated with mitosis at late G2 stage (Liao et al., 1995). Cenpq and Fam83d are required for chromosome congression (Bancroft et al., 2015; Wang et al., 2013). Cyclin B1 controls the G2/M transition phase during cell cycle (Petrachkova et al., 2019). Downregulation of these genes suggests that high dose of E + C may interfere normal cell division and in turn lead to cell dsfunction. Cytochrome P450 family enzymes are important proteins for drug-drug interaction (Manikandan and Nagini, 2018). Among them, Cyp1a2, Cyp2c7, Cyp3c9, and Cyp26a1 which were enriched in multiple metabolic processes was involved in DEGs of group E + Q compared with the control group. Another study also reported the changes of CYP1A and CYP26a after multiple antimicrobials intake (Qiu et al., 2020). These in all were evidence that the combined use of E + Q may interfere metabolite system.

In the evaluation of joint toxicity, it is necessary to evaluate mixtures of more than two drugs. In this study, we managed to obtain the specific effect that distinguishes between multiple mixed drugs and their subcomponents using WGCNA. The relative expression levels of the obtained hub genes were significantly different from that of the other six treatments, proving that WGCNA is suitable for analyzing mixtures with more than two drug components. In group E + C+Q, several immune regulation-related genes were GO enriched in the negative regulation of lymphocyte proliferation pathway, reaching a consensus with the abnormality of lymphocytes at 400 mg/(kg·BW) and 80 mg/(kg·BW). In group E + C+Q, the expression of Cyp2a2 and Cyp2c13 were downregulated while Cyp2c12 was surprisingly upregulated. Cyp2a2 and Cyp2c13 are considered only expressed in male rats whereas Cyp2c12, mainly regulated by growth hormone, is only expressed in female rats (Endo et al., 2005; Raish et al., 2017). This finding indicates E + C+Q might regulate these enzymes in a gender related pathway and in turn obtrude drug metabolism.

5. Conclusion

A 14-day short-term toxicity study showed that E + C+Q treatment induced severe hepatoxicity at the dose of 400 mg/(kg·BW) per drug. However, no obvious hepatoxicity impact was observed in combination

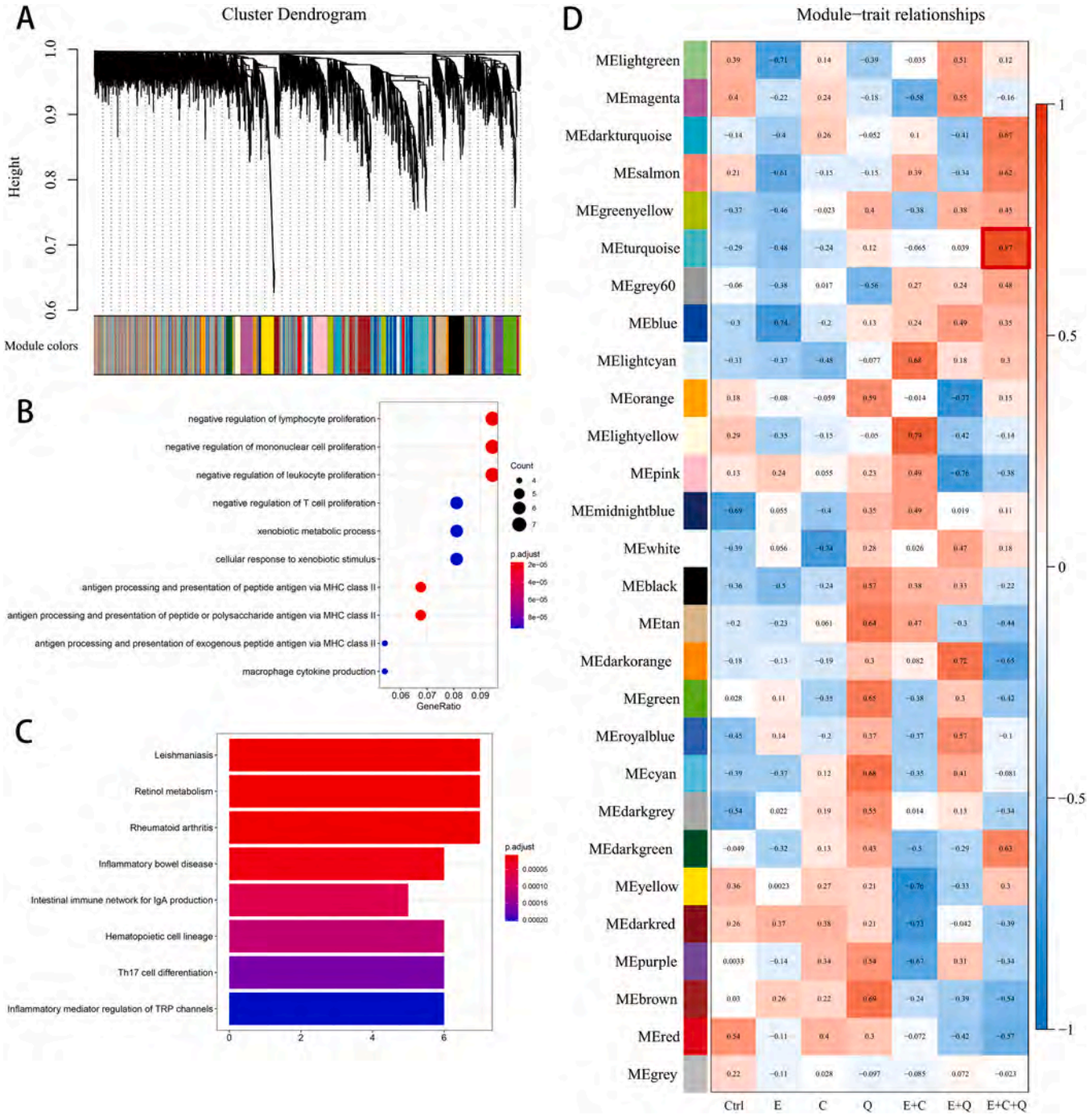

Fig. 4. (A) Cluster Dendrogram of top 5000 common genes for standard deviation in all samples (B) GO analysis results (biological process) of hub genes selected from MEturquoise module. (C) KEGG analysis results of hub genes selected from MEturquoise module. (D) Pearson correlation analysis results between each module and treatment.

E + C, E + Q, and single drugs. Transcriptome study revealed that E + C treatment had the potential to interfere with cell cycle, E + Q treatment disrupted the metabolic pathways. E + C+Q treatment interfered with both metabolic functions and immune functions. This study would enrich references for the risk assessment of antimicrobial mixtures.

CRediT authorship contribution statement

Yehui Luan: Conceptualization, Methodology, Writing - original draft preparation. **Junjie Zhao**: Investigation and Software **Hongfei Han:** Investigation. **Jianzhong Shen**: Resources. **Shusheng Tang**: Validation and Supervision. **Linli Cheng**: Writing - review & editing, Project administration and Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2021.112398.

References

Babaahmady, E., 2011. Toxicology of baytril (enrofloxacin). Afr. J. Pharm. Pharmacol. 5.
Bancroft, J., Auckland, P., Samora, C.P., McAinsh, A.D., 2015. Chromosome congression is promoted by CENP-Q- and CENP-E-dependent pathways. J. Cell Sci. 128, 171–184.
Cai, Y., Lee, W., Kwa, A.L., 2015. Polymyxin B versus colistin: an update. Expert Rev. Anti-Infect. Ther. 13, 1481–1497.
Chen, Q., Tang, S., Jin, X., Zou, J., Chen, K., Zhang, T., Xiao, X., 2009. Investigation of the genotoxicity of quinocetone, carbadox and olaquindox in vitro using Vero cells. Food Chem. Toxicol. 47, 328–334.
Das, U.N., 2007. Acetylcholinesterase and butyrylcholinesterase as possible markers of low-grade systemic inflammation. Med. Sci. Monit. 13, RA214–RA221.
Endo, M., Takahashi, Y., Sasaki, Y., Saito, T., Kamataki, T., 2005. Novel gender-related regulation of CYP2C12 gene expression in rats. Mol. Endocrinol. 19, 1181–1190.
Espersen, G.T., Stamp, I., Lidang Jensen, M., Elbaek, A., Ernst, E., Kahr, O., Grunnet, N., 1995. Lymphocyte redistribution in connection with physical activity in the rat. Acta Physiol. Scand. 155, 313–321.
Eu commission 2013. COMMISSION IMPLEMENTING REGULATION (EU) No 1057/2013 of 29 October 2013 - amending the Annex to Regulation (EU) No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, as regards the substance manganese carbonate Text with EEA relevance OJ L 288, 30.10.2013, p. 63–65.
Falagas, M., Kasiakou, S., 2006. Toxicity of polymyxins: a systematic review of the evidence from old and recent studies. Crit. Care 10, 27.
Gomes, M.P., Moreira Brito, J.C., Cristina Rocha, D., Navarro-Silva, M.A., Juneau, P., 2020. Individual and combined effects of amoxicillin, enrofloxacin, and oxytetracycline on Lemna minor physiology. Ecotoxicol. Environ. Saf. 203, 111025.
Grave, K., Torren-Edo, J., Mackay, D., 2010. Comparison of the sales of veterinary antibacterial agents between 10 European countries. J. Antimicrob. Chemother. 65, 2037–2040.
Grave, K., Greko, C., Kvaale, M.K., Torren-Edo, J., Mackay, D., Muller, A., Moulin, G., ESVAC, G., 2012. Sales of veterinary antibacterial agents in nine European countries during 2005-09: trends and patterns. J. Antimicrob. Chemother. 67, 3001–3008.
Huygens, J., Daeseleire, E., Mahillon, J., Van Elst, D., Decrop, J., Meirlaen, J., Dewulf, J., Heyndrickx, M., Rasschaert, G., 2021. Presence of antibiotic residues and antibiotic resistant bacteria in cattle manure intended for fertilization of agricultural fields: a one health perspective. Antibiotics 10, 410.
Jia, L.L., Zhong, Z.Y., Li, F., Ling, Z.L., Chen, Y., Zhao, W.M., Li, Y., Jiang, S.W., Xu, P., Yang, Y., Hu, M.Y., Liu, L., Liu, X.D., 2014. Aggravation of clozapine-induced hepatotoxicity by glycyrrhetinic acid in rats. J. Pharm. Sci. 124, 468–479.
Kim, D., Langmead, B., Salzberg, S.L., 2015. HISAT: a fast spliced aligner with low memory requirements. Nat. Methods 12, 357–360.
Klaudia, C., Alina, W., 2015. The influence of enrofloxacin, florfenicol, ceftiofur and E. coli LPS interaction on T and B cells subset in chicks. Vet. Res. Commun. 39, 53–60.
Langfelder, P., Horvath, S., 2008. WGCNA: an R package for weighted correlation network analysis. BMC Bioinform. 9, 559.
Li, Y., Jiang, M., Thunders, M., Ai, X., Qiu, J., 2018. Effect of enrofloxacin and roxarsone on CYP450s in pig. Res. Vet. Sci. 117, 97–98.
Li, Y., Zhuang, Y., Tian, W., Sun, L., 2020. In vivo acute and subacute toxicities of phenolic extract from rambutan (Nephelium lappaceum) peels by oral administration. Food Chem. 320, 126618.
Liao, H., Winkfein, R.J., Mack, G., Rattner, J.B., Yen, T.J., 1995. CENP-F is a protein of the nuclear matrix that assembles onto kinetochores at late G2 and is rapidly degraded after mitosis. J. Cell Biol. 130, 507–518.
Liao, Y., Smyth, G.K., Shi, W., 2019. The R package Rsubread is easier, faster, cheaper and better for alignment and quantification of RNA sequencing reads. Nucleic Acids Res. 47, 47.
Manikandan, P., Nagini, S., 2018. Cytochrome P450 structure, function and clinical significance: a review. Curr. Drug Targets 19, 38–54.
McEntaggart, K., et al., 2019. EU insights chemical mixtures awareness, understanding and risk perceptions. EFSA Support. Publ. 16.
Ministry of Agriculture and Rural Areas of China., et al. 2019. GB 31650–2019 National Food Safety Standard- Maximum Residue Limits for Veterinary Drugs in Foods.
Ozer, J., Ratner, M., Shaw, M., Bailey, W., Schomaker, S., 2008. The current state of serum biomarkers of hepatotoxicity. Toxicology 245, 194–205.
Petrachkova, T., Wortinger, L.A., Bard, A.J., Singh, J., Warga, R.M., Kane, D.A., 2019. Lack of cyclin B1 in zebrafish causes lengthening of G2 and M phases. Dev. Biol. 451, 167–179.
Qiu, W., Liu, X., Yang, F., Li, R., Xiong, Y., Fu, C., Li, G., Liu, S., Zheng, C., 2020. Single and joint toxic effects of four antibiotics on some metabolic pathways of zebrafish (Danio rerio) larvae. Sci. Total Environ. 716, 137062.
Raish, M., Ahmad, A., Alkharfy, K.M., Jan, B.L., Mohsin, K., Ahad, A., Al-Jenoobi, F.I., Al-Mohizea, A.M., 2017. Effects of Paeonia emodi on hepatic cytochrome P450 (CYP3A2 and CYP2C11) expression and pharmacokinetics of carbamazepine in rats. Biomed. Pharmacother. 90, 694–698.
Tong, F., Zhao, Y., Gu, X., Gu, C., Lee, C.C., 2015. Joint toxicity of tetracycline with copper(II) and cadmium(II) to Vibrio fischeri: effect of complexation reaction. Ecotoxicology 24, 346–355.
Van Boeckel, T.P., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A., Robinson, T.P., Teillant, A., Laxminarayan, R., 2015. Global trends in antimicrobial use in food animals. Proc. Natl. Acad. Sci. U.S.A. 112, 5649–5654.
Vancutsem, P.M., Babish, J.G., 1996. In vitro and in vivo study of the effects of enrofloxacin on hepatic cytochrome P-450. Potential Drug Interact. Vet. Hum. Toxicol. 38, 254–259.
Wang, X., Zhang, W., Wang, Y., Peng, D., Ihsan, A., Huang, X., Huang, L., Liu, Z., Dai, M., Zhou, W., Yuan, Z.H., 2010. Acute and sub-chronic oral toxicological evaluations of quinocetone in Wistar rats. Regul. Toxicol. Pharm. 58, 421–427.
Wang, Z., Liu, Y., Zhang, P., Zhang, W., Wang, W., Curr, K., Wei, G., Mao, J.H., 2013. FAM83D promotes cell proliferation and motility by downregulating tumor suppressor gene FBXW7. Oncotarget 4, 2476–2486.
Yang, X., Tang, S., Li, D., Li, B., Xiao, X., 2018. ROS-mediated oligomerization of VDAC2 is associated with quinocetone-induced apoptotic cell death. Toxicol. Vitr. 47, 195–206.
Yen, H.J., Lin, J.R., Yeh, Y.H., Horng, J.L., Lin, L.Y., 2021. Exposure to colistin impairs skin keratinocytes and lateral-line hair cells in zebrafish embryos. Chemosphere 263, 128364.
Yuan, G., Dai, S., Yin, Z., Lu, H., Jia, R., Xu, J., Song, X., Li, L., Shu, Y., Zhao, X., 2014. Toxicological assessment of combined lead and cadmium: acute and sub-chronic toxicity study in rats. Food Chem. Toxicol. 65, 260–268.