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Glucose-Induced Resistance to Ciprofloxacin and Erythromycin in *Enterococci*

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Abstract

Model ecosystems were established to study the effect of glucose on the resistant phenotype of water source *enterococcus* to erythromycin (ERY) and ciprofloxacin (CIP). The mechanism of ERY and CIP-resistant were studied as well. After the model ecosystem was established, the system were divided into six groups, with the addition of 0.02 g/L, 0.1 g/L, 0.4 g/L, 1.6 g/L, 3.2 g/L and 0 g/L sterilized glucose. Isolation time at 0 d, 1 d, 8 d, 16 d, 28 d, 40 d, 84 d and 140 d, *enterococci* present in the mud samples were evaluated for their sensitivities to CIP and ERY. The *ermB*, *mefA, gyrA* and *Esp* genes were detected, *gyrA* gene was sequenced and codons 83 and 87 were analyzed for mutations. Isolation rates were highest in 0.02 g/L kegs. CIP-resistant phenotype was correlated to the dosages of glucose. The incidence of *ermB*-positive strains of ERY-resistant *enterococci* (28 strains) was 63.6%, while 0% from ERY-sensitive *enterococcus* (16 strains). The incidence of *gyA*-positive strains of ERY-resistant *enterococci* (28 strains) was 63.6%. There were no mutations at codons 83 and 87 in the *gyrA* gene from 72 strains. Our study indicated that adding different doses of glucose caused enterococci resistance to CIP and ERY in different degrees. The more glucose added the greater CIP-resistance and resistant strains appeared relatively earlier. CIP-resistant strains were not caused by *Esp*, *mefA* and *gyrA* genes mutation. However, in the vast majority of cases, ERY-resistance was related to the ermB resistance gene.

Keywords: Glucose; *Enterococcus*; Model ecosystem; Antibiotic-resistance

Introduction

Enterococci can easily colonize the gastrointestinal tracts and survive on the surfaces [1]. Antibiotic resistance in the aquatic environment has attracted wide attentions. For instance, [2] monitored drug resistance in 103 Gram-negative oxytetracycline-resistant bacteria isolated from various sources of four freshwater salmon farms and found that most of the bacteria were resistant to ERY, chloramphenicol, florfenicol, amoxicillin, ampicillin, cephalexin, furazolidone, trimethoprim, and linezolid. Moreover, all linezolid-resistant isolates were also resistant to ampicillin and CIP [3]. Antibiotic resistance genes (ARGs) in aquatic environment and other environment have been widely studied because ARGs have the possibility be transferred to human beings [4]. Antibiotic resistance mechanisms in the aquatic environment or related resistance factors were seldom reported. In fact, the presence of antibiotics was not the prerequisite for the appearance of antibiotic resistance phenotypes in the environment. McPhearson et al. [5] suggested that the composition of fish feed was causally related to the elevated frequency of the resistance detected on the farms without recent application history of antimicrobial agents. Kapetanaki et al. [6] studied laboratory simulations of the environments in a marine cage farm described by [7] and proved that the anaerobic decomposition of fish feed in some marine mesocosm systems was a sufficient condition for the increase emergence in the resistance to oxytetracycline. Moreover, they demonstrated that the presence of oxytetracycline was not a necessary condition for such an increase. Vaughan et al. [8] observed the same antibiotic resistance processes in freshwater mesocosms. Yu et al. [9] studied the effects of the antibiotic administration mode on the antibiotic resistance in *enterococci faecalis* in aquatic ecosystems and found that the presence of feed residue in the aquatic system was the cause for the appearance and persistence of antibiotic-resistant bacteria in sediments. The impacts of non-antibiotic factors like nutrition factors on environmental antibiotic resistance have been widely studied. Early studies were mainly focused on the impact of the resistance to tetracyclines which were widely applied in aquaculture. Other antibiotics such as ciprofloxacin, erythromycin and florfenicol are also important for their wide applications and different resistance mechanisms. Glucose is a commonly used energy feed in aquaculture and is sufficient to satisfy the demands for bacterial growth. In this study, with *enterococci* as a bacterial indicator, we studied the effects of different glucose doses on the appearance of antibiotic resistance to CIP and ERY in the model ecosystem and explored the mechanisms of antibiotic resistant phenotypic changes.

Materials and Methods

Materials

Model Ecosystem: The model ecosystem was established in October 2014. Soil samples were collected from Fuzhou National Forest Park. The *enterococci* were underwent separation and purification from the soil samples, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI), and the broth microdilution method was used to determine the minimum inhibitory concentrations (MICs) of two types of antibiotics. The results showed that the soil sample *enterococci* were sensitive to CIP and ERY that can be used as soil in the ecosystem. The soil samples were uniformly placed in 12 sterile 2.8-cubic-decimeter plastic kegs. The sediment thickness in each keg was 5 cm. Two liters of tap water that had been left standing for a day were added to each of the 12 kegs. All the kegs reached the same water level and were cultured for 14 d under natural conditions.

Standard Antibiotics: ERY and CIP were purchased from Fujian province drug inspection (Fujian, China).

Reagents: OXOID CM0131 and OXOID CM0377 was purchased from OXOID Company (UK); MH broth was purchased from Beijing Double Spin Microbiological Media Products Factory (Beijing, China); PCR Amplification Kit was purchased from Shanghai Biological Engineering Company (Shanghai, China); Mini gel extraction kit was purchased from Omega company (USA).

Standard Strains: *E. faecalis* (ATCC 29212) was purchased from China Pharmaceutical and Biological Products Inspection (Beijing, China).

Methods

Model Ecosystem: After the establishment of the model ecosystem, sediments from the water-soil interface were collected using a homemade sample collection device and were considered day 0 samples. Then the model ecosystems were divided into six dose groups for testing. Sterilized glucose was added to the groups at concentrations of 0.02 g/L, 0.1 g/L, 0.4 g/L, 1.6 g/L, 3.2 g/L, and 0 g/L. Each dose group was created in duplicate. The model ecosystem was established during the summer, and the sampling time lasted until the late autumn. Over this period of time, due to climate changes, the water level of the model ecosystems was time varying. To avoid this effect, when the weather was sunny, tap water that had been standing for a day was added to re-establish the original water levels. The kegs were temporarily covered with lids during heavy rain. All kegs were monitored on a daily basis, and observations were recorded.

Sample Collection: Before adding sterile glucose, a homemade sample collector was used to collect a day 0 sample. Soil samples were collected and stored in sterile Eppendorf tubes. On days 0, 1, 8, 16, 28, 40, 86 and 140 after glucose addition, ecosystem sediment was collected and stored in sterile Eppendorf tubes. The sampling points were uniformly distributed over the surface of the entire system. Five samples were collected from each keg at each sampling time, for a total of ten samples for each treatment.

***Enterococci* Isolation, Purification, and Identification:** The collected soil samples were inoculated into test tubes with 2 mL sterile Mueller-Hinton broth (MH broth), fully vortexes, and incubated at 25°C for 18-24 h. After enrichment, the samples were inoculated on Oxoid CM0377, a selective medium for the isolation of enterococci, and incubated at 25°C for 48 h. Red or pink large colonies were observed growing in the culture medium. Typical colonies were picked, inoculated on enterococci nutrient agar (Oxoid CM0131), and incubated at 25°C for 18-24 h. Small, round, white colonies were selected for testing. Single enterococci colonies on nutrient agar medium were picked for inoculation into pH 9.6 MH broth; 6.5% sodium chloride MH broth and 0.1% methylene blue milk, and incubated at 25°C for 18-24 h. At the same time, single colonies were picked, inoculated into MH broth at 45°C, and cultured for 18-24 h. Bacterial growth was observed, and enterococci colonies were selected. The bacteria were identified by the principle of not exceeding one strain in each sample.

Enterococci Preservation: Isolation of *enterococci* been identified inoculated into test tubes with 2 mL sterile Mueller-Hinton broth (MH broth) and incubated at 26 °C for 18-24 h, then stored in sterile Eppendorf tubes mixed with 20% sterile glycerol, preserved these Eppendorf tubes in -20°C refrigerator.

***Enterococci* Sensitivity Test:** Antimicrobial susceptibility testing was performed according to the reference broth microdilution method, as recommended by CLSI (Clinical and Laboratory Standards Institute [CLSI, 2006a]). Susceptibility test results were interpreted using CLSI (2006b) breakpoint criteria for sensitive (S), intermediate (I) and resistant (R) in μg/mL were followed: (1) ERY: S≤0.5, 0.5≤I≤8, 8≤R; (2) CIP: S≤1, I=2, 4≤R.

Detection of Resistance Genes: The boiling method was used to extract bacterial DNA. To amplify the *ermB*, *mefA, Esp* and gyrA genes, previously published primers were used which were synthesized by the Shanghai Biological Engineering Company [10,11]. PCR was performed using a PCR kit (Takara, Dalian, China). The 30 μL reaction mixtures included 20 μM primers; 5 μL 10×PCR Buffer (Mg^{2+} Plus); 5 U/μL TaKaRa Taq 0.25 μL; dNTP Mixture 4 μL and 2 μL of 10ng/μL DNA sample; Fill with Double-distilled water. The PCR products were separated by electrophoresis in a 1% agarose gel containing 0.5% ethidium bromide. The target bands were analyzed densitometrically using a gel imaging system (model UVI Tec DBT 2.08, U.K. UVI Tec Company). The PCR-amplified products were sequenced by Takara Dalian.

The PCR conditions as followed: (1) the *gyrA* gene: initial denaturation at 94°C for 5 min followed by 35 cycles of 94°C for 30s, 54°C for 30s, 72°C for 30s and termination for 2 min at 72°C. (2) the *ermB* gene: initial denaturation at 93°C for 3 min followed by 35 cycles of 93°C for 1 min, 52°C for 1 min, 72°C for 5 min and termination for 5 min at 72°C. (3) the *mefA* gene: initial denaturation at 95°C for 2 min followed by 30 cycles of 95°C for 1 min, 56°C for 2 min, 72°C for 2 min and termination for 10 min at 72°C. (4) the *Esp* gene: initial denaturation at 95°C for 10 min followed by 30 cycles of 94°C for 45s, 63°C for 45s, 72°C for 60s and termination for 10 min at 72°C.

Statistical Analysis: Statistical analysis of the results of the antibiotic sensitivity tests was performed using the statistical software SPSS 13.0 (t-test).

Results

Changes in Model Ecosystem Appearances

Different doses of glucose showed significant impacts on the appearance of the model ecosystem. The phenomenon of slight turbidity appeared in the kegs with the glucose concentration above 0.4 g/L because a large quantity of microorganisms had been reproduced. On Day 16, the sediment surface turned lightly green in the 0.02 g/L and 0.1 g/L kegs. A red layer of oil-like substance emerged in the 1.6 g/L and 3.2 g/L kegs on Day 28 due to the algae from sediments. As shown in Table 1, a large number of microorganisms died and water was smelly because dissolved oxygen was consumed in the sample kegs from Day 1 to Day 140. Therefore, it is necessary to pay attention to reasonable feeding, increase feed efficiency, and control the food consumption in aquaculture.

Table 1 The effect of different concentrations glucose on the isolation rate of *enterococci*

Sampling time (d)	0	1	8	16	28	40	86	140
Added 0g glucose (%)	30	40	30	40	50	40	30	40
Added 0.02g glucose (%)	20	60	80	70	70	90	70	40
Added 0.1g glucose (%)	20	60	70	60	70	60	50	30
Added 0.4g glucose (%)	20	40	80	60	70	50	40	40
Added 1.6g glucose (%)	50	30	40	30	30	100	40	40
Added 3.2g glucose (%)	40	50	50	30	30	40	80	30

Impacts of Glucose Concentration on *Enterococci* CIP Resistance

Different doses of glucose had significantly different effects on *enterococci* CIP resistance. The antibiotic resistance of 33.3% occurred on Day 1 in the 0.1 g/L glucose kegs and the highest resistance rate reached 60% on Day 1 in 3.2 g/L kegs. In the 0.02 g/L glucose kegs, the resistant level reached 57.1% on Day 28. Subsequently, the resistant level gradually decreased. Only 40% intermediate strains emerged on Day 140 and no resistant isolate was obtained. The separation level of intermediate and the resistant level of 0% emerged in the 0.1 g/L glucose kegs on Day 8. Subsequently, the separation level gradually increased, then reached its maximum value of 42.9%, and then declined to 33.3% until Day 140. Resistant strains emerged in the 0.4 g/L and 1.6 g/L glucose kegs on Day 1 and the highest resistant levels in the 0.4 g/L and 1.6 g/L glucose kegs appeared on Day 16 and 28, respectively. Subsequently, the resistant level gradually declined. The resistant separation ratio appeared relatively earlier in the 3.2 g/L glucose kegs and the resistant level of 60% appeared on day 1 and then increased to 66.7% on Day 16. Subsequently, the resistant level decreased obviously because dissolved oxygen declined. The resistant level of 12.5% and the intermediate level of 37.5% appeared on Day 86 and disappeared on Day 140. The results are shown in Table 2.

Table2 The effects of different doses of glucose additives on the isolation of CIP resistant *enterococci*

Additive dose (g/L)		0	0.02	0.1	0.4	1.6	3.2
0d	S (%)	3	2	2	2	5	4
	I (%)	33.3	0	0	0	0	0
	R (%)	0	0	0	0	0	0
	(%)	4	6	6	4	3	5
1d	I (%)	0	0	33.3	50	0	0
8d	R (%)	0	0	33.3	25	33.3	60
	S (%)	3	8	7	8	4	5
	I (%)	0	62.5	0	50	0	20
	R (%)	0	25	0	25	25	60
16d	S (%)	4	7	6	6	3	3
	I (%)	0	14.3	16.7	16.7	33.3	0
	R (%)	0	28.6	16.7	33.3	66.7	66.7
	S (%)	5	7	7	7	3	3
28d	I (%)	20	0	42.9	14.3	33.3	0
40d	R (%)	0	57.1	42.9	14.3	66.7	33.3
	S (%)	4	9	6	5	10	4
	I (%)	0	22.2	16.7	0	30	0
	R(%)	0	22.2	33.3	0	70	0
	S (%)	3	7	5	4	4	8
86d	I (%)	0	14.3	20	25	0	37.5
	R (%)	0	0	20	0	25	12.5
	S(%)	4	5	3	4	6	3
140d	I (%)	25	40	0	50	16.7	0
	R(%)	0	0	33.3	25	16.7	0

Note: S(%)indicates the ratio of isolated *enterococci*, I (%) indicates the ratio of intermediate resistant *enterococci*, R (%) indicates the ratio of resistant *enterococci*

Effects of Glucose Concentration on *Enterococci* ERY Resistance

Different doses of glucose had different impacts on ERY resistance significantly. Intermediate levels of 20% and 25% in the 0 g/L glucose kegs occurred on Day 28 and 40, respectively. Intermediate and resistant strains emerged in the 0.02 g/L glucose kegs on Day 8 at the levels of 12.5% and 25%, respectively. Resistant and intermediate isolates increased, but the resistance declined to 20% on Day 140. ERY-resistance strains in the 0.1 g/L glucose kegs appeared on Day 1 with a resistant level of 50%, but the ratio of resistance declined from Day 8 to Day 86 and immediately increased to 66.7% on Day 140. Intermediate and resistant strains in the 0.4 g/L glucose kegs emerged on Day 8 and continued to increase. Then the resistance level reached the highest level of 50% on Day 140. Intermediate and resistant isolates in the 1.6 g/L and 3.2 g/L glucose kegs emerged on Day 8 and Day 1, respectively. The intermediate ratio of 66.6% occurred until Day 28 and 66.7%. The resistance ratios of 33.3% and 60% occurred on Day 28 and Day 1. However, the resistant level was decreased to 0% on Day 140. These results are shown in Table 3.

Table 3 The effects of different doses of glucose additives on the isolation of ERY resistant *enterococci*

Addictive does(g/L)		0	0.02	0.1	0.4	1.6	3.2
0d	S (%)	3	2	2	2	5	4
	I (%)	0	0	0	0	0	0
	R (%)	0	0	0	0	0	0
	S (%)	4	6	6	4	3	5
1d	I (%)	0	0	16.7	0	0	20
	R (%)	0	0	50.0	0	0	60
	S (%)	3	8	7	8	4	5
8d	I (%)	0	12.5	57.1	12.5	25	0
	R (%)	0	25	0	25	25	40
	S (%)	4	7	6	6	3	3
16d	I (%)	0	28.6	16.7	33.3	33.3	33.3
	R (%)	0	42.9	33.3	16.7	0	33.3
	S (%)	5	7	7	7	3	3
28d	I (%)	20	14.3	28.6	28.6	66.6	66.7
	R (%)	0	28.6	28.6	14.3	33.3	0
	S (%)	4	9	6	5	10	4
40d	I (%)	25	44.4	33.3	0	0	0
	R (%)	0	44.4	33.3	20	10	25
	S (%)	3	7	5	4	4	8
86d	I (%)	0	42.9	20	25	25	25
	R (%)	0	14.3	20	25	25	37.5
	S (%)	4	5	3	4	6	3
140d	I (%)	0	40	33.3	25	16.7	33.3
	R (%)	0	20	66.7	50	0	0

Note: I (%) indicates the ratio of intermediate resistant *enterococci*, R (%) indicates the ratio of resistant *enterococci*

gyrA Test Results and Sequence Analysis

The incidence of *gyrA*-positive strains among the 72 *enterococcus* isolates from the model ecosystem was 83.3% (60/72). The incidence of gyrA in the CIP-resistant *enterococci* was 94.2% (49/52). The incidence of *gyrA* in the CIP-sensitive *enterococci* was 80% (16/20). As shown in Figure 1, samples 1, 2, 3, 7, 8 and 9 are *gyrA*-positive. In Figure 2, the SeqMan program was used in the Lasergene 7.1 suite for splicing, and BLAST analysis was performed with the sequences obtained after splicing. When compared to the published *gyrA* sequence (GenBank No: L29417.1), no mutation at codon 83 or 87 was obtained.

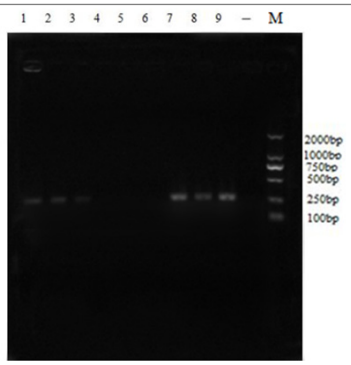

Fig. 1. The test results of *gyrA* gene of *enterococcus*.
M: DNA marker. —: Negative control (H2O). 1 2 3 4 5 6 7 8 9: samples

ERY Resistance Gene Test Results

Among the 44 *enterococci* strains from the model ecosystem that were resistant to ERY, the incidence of ermB-positive strains was 63.6% (28/44) and there were 16 *enterococci* ermB-negative strains. Among the 28 ERY-resistant *enterococci*, 24 strains were resistant to ERY at the concentrations of 256 μg/mL and 128 μg/mL. These results indicated that the appearance of ERY-resistant *enterococcus* showed the strong correlation with the appearance of ermB gene. All these 16 strains sensitive to ERY were erm-negative (Figure 3). Only one mefA-positive strain emerged among these isolates that were sensitive or intermediate to ERY.

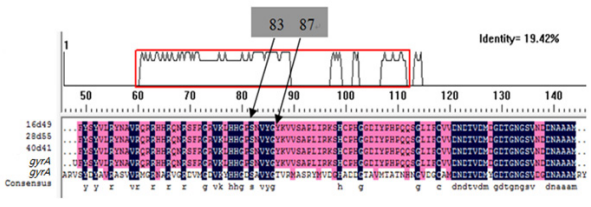

Fig. 2. The analysis results of *gyrA* sequence

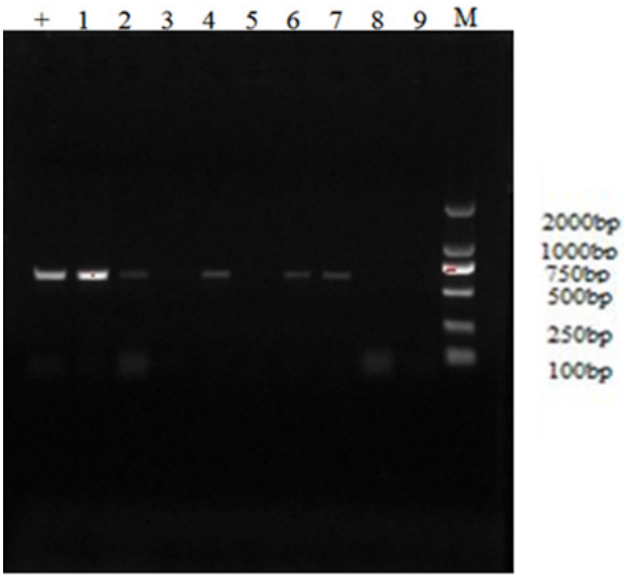

Fig. 3. The test result of *ermB* of erythromycin resistance gene.
M: DNA marker. +: Positive control (Strain of Laboratory Saving). 1 2 3 4 5 6 7 8 9: samples

Esp Gene Test Results

The incidence of *ermB*-positive strains was 0% among 96 *enterococci* strains which were resistant to ERY and CIP. With *Esp*-positive *enterococci* strains as the control, target bands at 954 bp were observed in electrophoresis.

Discussion

Correlation of Glucose Concentration with the *Enterococci* isolation rate

As shown in Table 1, the glucose dose was positively correlated with *Enterococci* isolation rate. *Enterococci* isolation rate in the 0.02 g/L glucose keg was increased from 20% to 90%. Isolation rates increased after glucose was added in all the kegs before Day 86 and declined significantly hereafter because the model ecosystem collapsed from excess nutrition and dissolved oxygen decreased greatly due to eutrophication of water. Furthermore, the ambient temperature on Day 140 in February was approximately 0 °C, which was unsuitable for *enterococci* growth.

Correlation of Glucose Concentration with the Appearance and Persistence of Resistance Phenotypes

It is feasible to study the influences of glucose dose on the drug resistance of *Enterococci* with our model system of bacterial growth. The experimental results showed that the long enough cultivation time would necessarily lead to the appearance of antibiotic-resistant bacteria and that the resistant levels of these bacteria were positively correlated to the dose of glucose. Huang et al. [12] found that bacteria cultured in a nutrient-rich medium were less sensitive to quaternary ammonium disinfectant and pointed out that when the nutrient concentration increased within a certain range, the MIC values of bacteria to disinfectants also increased. This phenomenon was also observed in this study. The relationship between eutrophication and the drug resistance is worthy further exploration.

In this study, the CIP-resistance rate was the highest (66.7%), the higher glucose addition led to the higher CIP-resistance rate. Different doses of glucose had significant impacts on ERY-resistant *enterococci* in this model ecosystem. Resistant and intermediate stains were isolated on Day 1. We concluded that the isolation rates of intermediate strains were increased along with the resistance. As a whole, the amount of ERY-resistant *enterococci* was relatively less than that of CIP-resistant *enterococci* in early phases. The effects of different glucose doses on ERY-resistant *enterococci* were not significant.

Meanwhile, the ampicillin or vancomycin resistance was not changed. This phenomenon indicated that an increase in nutrition situation in the model ecosystem led to bacterial resistance to some antibiotics. However, all antibiotic resistance phenotypes were not changed. This phenomenon may indicate its underlying mechanisms.

Emergence of Intermediate Resistant Strains

ERY-intermediate *enterococci* appeared along with RY-resistant *enterococci* (on Day 1). The glucose dose was not exactly proportional to the level of phenotypic impact. Under the small glucose dose, resistant strains were not obtained in the early stages of the experiment. In the 3.2 g/L and 1.6 g/L glucose kegs, intermediate and resistant strains were observed on Day 1 and Day 8, respectively. This observation indicated that the induction of ERY-resistant *enterococci* by glucose required a particular course. Large doses induced the resistance within a short time, and small dose induced the resistance over a longer time. The results indicate that intermediate strains may further develop into antibiotic-resistant strains. Generally, the mechanisms in the resistant strains are more complicated than those in the intermediate strains [13]. The results indicated that under the simple mechanism, the accumulated changes might lead to the higher resistance level in the model ecosystems.

Possible Mechanism for the Appearance of the CIP Resistance Phenotype

The *gyrA* gene was detected in 72 strains of CIP-resistant *enterococci* and 20 strains of CIP-sensitive *enterococci*. According to the gene sequence analysis results, codons 83 or 87 in the *gyrA* gene QRDR showed no mutation, indicating that CIP resistance was unrelated to the *gyrA* gene mutation in the ecosystem. Quinolone antibiotic resistance was mainly caused by reduced antibiotic concentrations in vivo and by *gyrA* gene and *parC* gene mutations [14,15]. The active efflux of antibiotics and changes in the permeability of bacterial cell walls could reduce antibiotic concentrations in vivo. Therefore, it is necessary to further study the detected *erfAB* gene, which is the most important factor for the resistance to CIP and other Quinolone antibiotic [16-18]. Previous study showed that glucose supplementation enhanced the biofilm formation among *E. faecalis* [19]. Yu et al. [20] found that *enterococci* resistance could be induced by multi-nutrition after adding cornmeal in model ecosystems. Moreover, resistant genotypic changes are not required in some resistant phenotypic changes. Therefore, it is necessary to study the mechanisms of the emergence of CIP-resistant *enterococci* induced by glucose, such as the correlations between environmental biofilms and antibiotic resistance phenotypes as well as nutrition factors.

Possible Mechanisms for the Appearance of the ERY Resistance Phenotype

The rate of *ermB* gene (63.6%) was detected in strains of ERY-resistant *enterococci*, but *mefA* gene detection was not evident, indicating that the resistance of waterborne *enterococci* to ERY induced by glucose addition was related to the appearance of *ermB* other than *mefA*. The *erm* genes mediated the host resistance to the macrolide-lincosamide-streptogramine B (MLSB) antibiotics. A methylase encoded by *erm* gives rise to a dimethylation of a specific adenine base in 23S ribosomal RNA [20]. The expression of *erm* can be either inducible or constitutive depending on the upstream regulatory region of the methylase structural genes. Two major determinants for MLSB resistance in *enterococci* are *ermB* and *ermA* [20]. The mefA gene codes an efflux protein that conveys resistance to 14- and 15-membered macrolides [21]. The *ermB* resistance gene was detected in most of the ERY-resistant *enterococci* isolated from the model ecosystem, but *mefA* resistance genes were not detected, indicating that glucose-induced ERY resistance in waterborne *enterococci* was related to the *ermB* resistance gene other than the *mefA* resistance gene. In this model ecosystem, the detection of ermA gene and the origin of the *ermB* resistance gene are worth further exploration.

Conclusions

Our study indicated that waterborne *enterococci* antibiotics resistance was caused by glucose addition. Different glucose doses caused *enterococci* resistance to CIP and ERY in different degrees. The higher glucose dose led to the stronger CIP resistance and the earlier appearance of resistant strains. CIP-resistant strains isolated from the model ecosystem were not caused by the mutations in *Esp*, *mefA*, and *gyrA* genes. However, in the vast majority of cases, ERY-resistance was related to the *ermB* resistance gene.

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