朱鹮大肠杆菌分离鉴定及耐药性与喹诺酮类耐药基因分子特征分析

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摘　要：【目的】掌握浙江省德清县驯养朱鹮大肠杆菌的耐药性、耐药基因与分子特征，为治疗朱鹮大肠杆菌感染提供基础数据。【方法】采集该地驯养朱鹮新鲜粪便样本，采用分离培养、形态观察、生化鉴定及 16S rDNA 序列分析鉴定大肠杆菌；进而采用肉汤稀释法检测环丙沙星、庆大霉素、丁胺卡那等 7 种抗菌药对源自不同朱鹮的大肠杆菌分离株菌株的最小抑菌浓度（minimal inhibitory concentration, MIC）；选择代表性菌株，通过 PCR 和测序鉴定其携带 *qnrS*1、*gyrA*、*gyrB* 等 9 种喹诺酮类耐药基因的情况，并分析其蛋白关键氨基酸位点突变与耐药性的关系，采用接合转移分析耐药质粒水平转移情况及其与耐药性的关系，应用卡方检验（Chi-square test）和费歇尔精确检验（Fisher exact test）分析分离株耐药表型与朱鹮年龄、耐药基因的相关性。【结果】本研究共采集了 98 只朱鹮粪便，经分离鉴定均获得了大肠杆菌；源自不同朱鹮的 98 株大肠杆菌对喹诺酮类环丙沙星呈现高度耐药（耐药率为 65.3%，64/98），对其余 6 种药物高度敏感（敏感率均＞90%），朱鹮年龄与环丙沙星耐药性极显著相关（*P*＜0.01）；选取的 31 株分离株喹诺酮类耐药基因 *marR*、*gyrA*、*parC* 和 *gyrB* 的突变株占比在 3.2%～80.6%之间、携带质粒耐药基因 *qnrS*1 的菌株占比 22.6%（7/31）；耐药基因阳性菌株占比 93.5%（29/31）并分布于 6 个耐药基因型，其中，*marR/gyrA/parC* 10 株、*marR/qnrS*1 6 株、*marR* 6 株、*gyrA/parC* 3 株、*marR/gyrA* 3 株、*marR/gyrB/qnrS*1 1 株；环丙沙星耐药表型与耐药基因显著相关（*P*＝0.026），*gyrA* 和 *parC* 单基因或联合突变均与喹诺酮耐药显著相关（*P*＝0.038）；大肠杆菌分离株 *qnrS*1 一次接合成功率为 28.6%(2/7)，并可导致受体菌对喹诺酮类药物的耐药性增加。【结论】本研究鉴定了朱鹮群体大肠杆菌耐药性，并明确了朱鹮源大肠杆菌对喹诺酮类耐药的主要原因是 *gyrA* 和 *parC* 单基因或联合突变并携带可水平转移的耐药基因 *qnrS*1，本研究结果为人工驯养朱鹮救护和耐药性监测提供参考依据，提示应重视朱鹮大肠杆菌对喹诺酮类药物的耐药性。

关键词：朱鹮；喹诺酮；耐药基因；分子特征

Isolation and Molecular Characteristics of Drug Resistance and Quinolone Resistance Genes in Escherichia coli of *Nipponia nippon*

Abstract:【Objective】The experiment aims to master the drug resistance, resistance genes, and molecular characteristics of *Escherichia coli* (*E. coli*) in domesticated *Nipponia nippon* in Deqing county, Zhejiang province, and provide basic data for the treatment of *E. coli* infection in *Nipponia nippon*.【Method】Fresh feces of *Nipponia*

nippon were collected and *E. coli* isolates were identified by using bacteria isolation and culture, biochemical characteristics and 16S rDNA sequencing. Then the broth dilution method was used to detect the minimum inhibitory concentration (MIC) of seven kinds of antimicrobial agents, including ciprofloxacin, gentamicin, and amikacin, against *E. coli* isolates from different *Nipponia Nippon*.

Nine quinolone resistance genes such as *qnrS*1, *gyrA* and *gyrB* of the respective strains were identified by PCR and sequencing, and the relationship between the mutation of the amino acid and drug resistance was analyzed. The horizontal transfer of plasmids carrying resistance genes were detected by conjugation experiments, and drug resistance were determined. The association between host's age, drug resistance-genes and drug resistance were analyzed by using Chi-square test and Fisher exact test. 【Result】A total of 98 *Nipponia nippon* feces were collected and all were isolated and identified with *E. coli* strains. Of which, 98 isolates from different captive *Nipponia nippon* were highly resistant to quinolone ciprofloxacin (65.3%, 4/98), and highly sensitive to the remaining 6 drugs (sensitivity rates are above 90%). The age of *Nipponia nippon* was highly significantly correlated with ciprofloxacin resistance ($P < 0.01$). Thirty-one *E. coli* strains isolated in this study were selected for resistance genes detection. The prevalence of mutations in the quinolone resistant genes *marR*, *gyrA*, *parC*, and *gyrB* in 31 selected isolates ranged from 3.2% to 80.6%, and the proportion of strains carrying the plasmid resistant gene *qnrS*1 was 22.6% (7/31). The isolates carrying one or more resistance genes accounted for 93.5% (29/31), and were distributed in six drug resistance genotypes, including 10 strains of *marR/gyrA/parC* genes, 6 strains of *marR/qnrS*1 genes, 6 strains of *marR* gene, 3 strains of *gyrA/parC* genes, 3 strains of *marR/gyrA* genes, and 1 strain of *marR/gyrB/qnrS*1 genes. The ciprofloxacin resistance phenotype was significantly correlated with drug resistance genotype ($P = 0.026$). Both monogenic and combined mutations of the *gyrA and parC* genes were related to quinolone resistance ($P = 0.038$). The conjugation experiment results showed that the transfer success rate of the *qnrS*1 resistance gene carried by the *E. coli* isolates in this study was 28.6% (2/7) in one conjugation event, which could lead to an increase in quinolone resistance in the recipient bacteria. 【Conclusion】 This study identified the antibiotic resistance of *E. coli* from *Nipponia nippon*, and identified that the main cause of quinolone resistance in *Nipponia nippon* was single or combined mutation of *gyrA* and *parC* genes, carrying the horizontally transferable resistance gene *qnrS*1. This study provided a reference for rescuing *Nipponia nippon* and a baseline data for quinolone resistance surveillance of *Nipponia nippon*.

Key words: *Nipponia nippon*; quinolone; drug resistance gene; molecular characteristic