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Bacteriology

Investigation and characterization of β-lactam resistance in *Escherichia coli* strains isolated from bamboo rats (*Rhizomys sinensis*) in Zhejiang province, China

Hui ZHANG[2)], Kun LI[2)], Yajing WANG[2)], Mujeeb Ur REHMAN[2)], Yijiang LIU[1)], Junjie JIN[1)], Junping PENG[4)], Fazul NABI[2)], Khalid MEHMOOD[2,5)], Houqiang LUO[1,2)]* and Jiaxiang WANG[3)]*

[1)]College of Animal Science, Wenzhou Vocational College of Science and Technology, Wenzhou 325006, People's Republic of China
[2)]College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, People's Republic of China
[3)]College of Animal Science, Yangtze University, Jingzhou, People's Republic of China
[4)]China Agricultural university, College of Veterinary Medicine, Beijing 100083, People's Republic of China
[5)]University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, 63100, Pakistan

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ABSTRACT. This study was undertaken to investigate drug resistance in *Escherichia coli* (*E. coli*) strains isolated from bamboo rats in Zhejiang province of China. One hundred and fifty-four *E. coli* strains were isolated from dead bamboo rats. Polymerase chain reaction (PCR) was used to detect the representative genes encoding resistance to commonly used β-lactam antibiotics. Highest resistance was observed for cefradine (24.03%), followed by penicillin (20.78%) and ceftazidime (20.13%). The isolation rates of β-lactam resistance genes were 53.25, 48.70, 15.58 and 14.29% for *bla* $_{TEM}$, *bla* $_{CTX-M}$, *bla* $_{OXA}$ and *bla* $_{SHV}$, respectively, while 62 (40.26%) *E. coli* isolates harbored multiple β-lactam resistance genes. These results also suggested that long term use of these antibiotics leads to antibimicrobial resistance. We believe that this study will provide a guideline for veterinarians and a research basis for examining resistance-encoding genes in other food animals like bamboo rats.

KEY WORDS: bamboo rat, β-lactam, *Escherichia coli*, genotypes, resistance

Escherichia coli is a commensal bacterium and opportunistic pathogen that is commonly found in the intestinal tracts of animals and humans [9]. Some pathogenic serotypes of *E. coli* can cause severe diarrhea, dehydration, sepsis, and even death. Therefore, *E. coli* is a serious threat to public health [15]. Although vaccines have been developed for preventing this infection, antimicrobial treatment is considered to be the most effective method for treating this disease. In the past few decades, β-lactam antibiotics such as penicillin, ampicillin, and aminopenicillins have become the most important antimicrobial agents for preventing colibacillosis [20]. However, the drug resistance of *E. coli* to this class of antibiotics is a major problem. With the widespread use of third-generation cephalosporins, broad-spectrum cephalosporin-resistant super microbes are evolving rapidly and are being constantly reported [1, 2]. Certain broad-spectrum cephalosporins have been approved for veterinarian use in China. Zhejiang, a developed province in China, has one of the important breeding industries of Bamboo rats. However, with the widespread application of β-lactam antibiotics, the main genotype of extended spectyum β-lactamase (ESBLs) encoding *bla* $_{TEM}$, *bla* $_{SHV}$, *bla* $_{CTX-M}$ and *bla* $_{OXA}$ were detected in *E. coli* in different animals [12].

Bamboo rats are mammals of subfamily rhizomyinae, genus cannomys. In China, bamboo rats are important sources of income for local residents since 1990 because of their high protein content [10]. However, recently, bamboo rats were identified as the cause of severe clinical diarrhea in Zhejiang, and treatment with β-lactam antibiotics was not found to be effective. Therefore, to understand the β-lactam resistance mechanism in bamboo rats and to provide a basis for reasonable clinical medication, we investigated the drug resistance pattern of *E. coli* by targeting *bla* $_{TEM}$, *bla* $_{SHV}$, *bla* $_{CTM-M}$ and *bla* $_{OXA}$ using PCR.

*Correspondence to: Luo, H.: chviolet1984@sina.com, Wang, J.: wangjiaxiang1109@163.com

Table 1. Drug susceptibility testing results of *E. coli* isolated from bamboo rats (n=154)

Antimicrobial agents (μg)	Resistant (%)	Intermediate (%)	Sensitive (%)
Ampicillin (10)	23 (14.94)	8 (5.19)	123 (79.87)
Amoxicillin/Clavulanic acid (20/10)	6 (3.90)	3 (1.94)	145 (94.16)
Penicillin (10)	32 (20.78)	0	122 (79.22)
Cephalexin (30)	24 (15.58)	2 (1.30)	128 (83.12)
Cefradine (30)	37 (24.03)	7 (4.55)	110 (71.42)
Cefazolin (30)	19 (12.34)	6 (3.90)	129 (83.76)
Ceftazidime (30)	31 (20.13)	0	123 (79.87)
Cefuroxime (30)	16 (10.39)	1 (0.65)	137 (88.96)
Ceftriaxone (30)	21 (13.64)	1 (0.65)	132 (85.71)
Cefoxitin (30)	27 (17.53)	0	127 (82.47)

MATERIALS AND METHODS

Animal

Chinese bamboo rats (Rhizomys sinensis) were collected from different breeding farms in the Wenzhou prefecture in south Zhejiang province of China between June 2015 and March 2016. The animals were kept in cages (well-supplied with food and water) with nesting materials on the solid floor for all natural activities. The housing rooms were well ventilated with ideal temperature, light schedule, and humidity.

Sample collection

One hundred and eighty adult (all samples were handled randomly irrespective of the gender) dead Chinese bamboo rats were necropsied, and the liver and other related pathological organs and feces were collected. All animals were suffering from diarrhea although they were reared as a food source. All samples were stored at 4°C and transported on ice to the Wenzhou Vocational College of Science and Technology for further experiments.

Isolation and identification of bacteria

Fecal samples were stored in 1 m*l* nutrient broth and were shifted to thermostatic cultivation at 37°C for 24 hr for enriching the organisms. The organisms were cross-inoculated in MacConkey medium, and a single pink colored colony was picked and purified on MacConkey agar (Hangzhou Microbial Reagent Co., Ltd., Hangzhou, China). Pink colored colonies were picked and inoculated on eosin methelene blue agar (EMB) (Hangzhou Microbial Reagent Co., Ltd., Hangzhou, China). For further validation, several biochemical tests (urease production, catalase test, motility test, voges-proskauer test, indole production assay, carbohydrate fermentation tests, methyl red and citrate utilization tests) were performed to identify *E. coli* strains. For species identification, 16S rDNA sequencing (Using universal primers) was performed as suggested by Edward and Wang [4, 16].

Antimicrobial susceptibility testing

Drug susceptibility was detected using the Kirby-Bauer disc diffusion method as recommended by the Clinical and Laboratory Standards Institute [3]. The following drugs were used: ampicillin, amoxicillin/clavulanic acid, penicillin, cephalexin, cefradine, cefazolin, ceftazidime, cefuroxime, ceftriaxone, and cefoxitin. The standard bacterial strain ATCC25922 was used as the positive control while clear broth was used as the negative control. Three types of susceptibility, i.e. resistance (R), intermediate (I), and sensitive (S) were recorded according to the criteria specified by the Clinical Laboratory Standards Committee (Clinical and Laboratory Standards Institute, recommended by CLSI).

PCR amplification of β-lactam resistance genes

Primers were designed as previously described [5, 6, 11] and synthesized by the Wuhan Qingke Co., Ltd. (Wuhan, China). Bacterial chromosomal DNA was extracted by the boiling method [19]. PCR was performed in a thermal cycler (Applied Biosystem, Foster City, CA, U.S.A.) using PCR kits (Takara, Dalian, China) according to the manufacturer's instructions. The PCR reaction was performed in a 25 μl mixture containing 13 μl 2 ×Taq PCR master mix, 1 μl of each primer, 8 μl double distilled H_2O, and 2 μl sample. The PCR reaction was performed following standard protocol [19]. The PCR products were electrophoresed on a 1.5% (w/v) agarose gel and observed using a gel imaging system (Gene Genius BioImaging System, U.K.).

RESULTS

Isolation, culturing, and identification of E. coli

Altogether, 154 isolates were identified as *E. coli* by biochemical tests and 16S rDNA sequencing analysis. The isolation rate in male and female animals was 69 (44.81%) and 85 (55.19%), respectively.

Table 2. β-lactam resistance genes in *E. coli* isolates

β-lactam resistance gene	No. samples	No. positive	Positive rate (%)
bla $_{TEM}$	154	82	53.25
bla $_{CTX-M}$	154	75	48.70
bla $_{OXA}$	154	24	15.58
bla $_{SHV}$	154	22	14.29

Table 3. Detection of multiple β-lactam resistance genes in *E. coli* isolates

No. positive isolates	Drug resistant phenotype (n)	Commensal β-lactam resistant genes	Positive rate (%) of resistant genes
46	A, C, D, E, G (28)	-	29.87
26	A, C, D, G, H (35)	*bla* $_{TEM}$	16.88
18	B, C, H, I, J (32)	*bla* $_{CTX-M}$	11.69
2	A, C (2)	*bla* $_{SHV}$	1.30
21	A, B, C, D, E, G, I, J (41)	*bla* $_{TEM}$ + *bla* $_{CTX-M}$	13.64
3	F, H (3)	*bla* $_{TEM}$ + *bla* $_{SHV}$	1.95
5	B, C (3)	*bla* $_{OXA}$ + *bla* $_{CTX-M}$	3.25
1	A (2)	*bla* $_{CTX-M}$ + *bla* $_{SHV}$	0.65
16	C, D, E, G, H, I, J (48)	*bla* $_{TEM}$ + *bla* $_{OXA}$ + *bla* $_{CTX-M}$	10.39
2	B, C (2)	*bla* $_{TEM}$ + *bla* $_{OXA}$ + *bla* $_{SHV}$	1.30
13	A, C, D, E, G, I, J (39)	*bla* $_{TEM}$ + *bla* $_{CTX-M}$ + *bla* $_{SHV}$	8.44
1	B (1)	*bla* $_{TEM}$ + *bla* $_{OXA}$ + *bla* $_{CTX-M}$ + *bla* $_{SHV}$	0.65

A: Ampicillin; B: Amoxicillin/Clavulanic acid; C: Penicillin; D: Cephalexin; E: Cefradine; F: Cefazolin; G: Ceftazidime; H: Cefuroxime; I: Ceftriaxone; J: Cefoxitin.

Drug resistance of E. coli isolates

The β-lactam resistance of the *E. coli* isolates is listed in Table 1. The highest resistance was observed for cefradine (24.03%), followed by penicillin (20.78%) and ceftazidime (20.13%). For other antibiotics, including cefoxitin, cephalexin, ampicillin, ceftriaxone, cefazolin, cefuroxime, and amoxicillin/clavulanic acid, the frequencies of resistance were 17.53, 15.58, 14.94, 13.64, 12.34, 10.39 and 3.90%, respectively (less than 20%).

PCR-mediated detection of drug resistance genes

The β-lactam resistance genes were amplified from 154 *E. coli* isolates recovered from bamboo rats using PCR. The prevalence rates were 53.25, 48.70, 15.58 and 14.29% for *bla* $_{TEM}$, *bla* $_{CTX-M}$, *bla* $_{OXA}$ and *bla* $_{SHV}$, respectively (Table 2).

Distribution of multiple β-lactam resistance genes in E. coli isolated from bamboo rats

Most of the *E. coli* isolates harbored multiple β-lactam resistance genes (Table 3). Out of 154 isolates, 108 (68.18%) had at least one β-lactam resistance gene with frequency distribution of 46 (29.87%), 30 (19.48%), 31 (20.13%), and 1 (0.65%). Conversely, 46 (29.87%) isolates did not carry any β-lactam resistance genes.

DISCUSSION

Resistance to extended-spectrum β-lactam antimicrobials in the Enterobacteriaceae family is an emergent global problem since it was first identified in *Klebsiella pneumoniae* twenty years ago [7]. In the last few decades, extended-spectrum β-lactamase-producing *E. coli* strains have been reported in many countries, especially China [17, 18]. Due to the widespread use of third-generation cephalosporins, *E. coli* strains resistant to ESBLs are of concern to the scientific community [18]. Several recent studies reported β-lactam resistance in animals and humans [8, 9, 15]. However, to the best of our knowledge, no information is available regarding ESBL-producing *E. coli* strains in bamboo rats, especially in China, where it is widely used as food. This study was designed to assess the drug susceptibility and presence of ESBL-producing *E. coli* strains in Chinese bamboo rats.

In this study, the rates of β-lactam resistance were high in *E. coli* isolates, which indicates a serious situation in bamboo rats. The prevalence of *bla* $_{TEM}$, *bla* $_{CTX-M}$, *bla* $_{OXA}$ and *bla* $_{SHV}$ observed in this study corroborated the results of a previous study [17]. Our results indicated that *bla* $_{TEM}$ and *bla* $_{CTX-M}$ are the key β-lactam resistance genes in bamboo rats. Meanwhile, *E. coli* producing extended-spectrum β-lactamases are of considerable concern because β-lactams are commonly used for the treatment of colibacillosis and other bacterial infections. This study also suggests that long-term irrational use of β-lactam antibiotics is the main cause of drug resistance in bamboo rats.

Reports mention different genotypes of β-lactam resistance genes, including *bla* $_{KPC}$, *bla* $_{TEM}$, *bla* $_{CTX-M}$, *bla* $_{SHV}$ and *bla* $_{OXA}$, etc.; however, these genotypes vary with geographical regions [13, 14, 19]. In this study, the main genotypes observed were

bla $_{TEM}$ and *bla* $_{CTX-M}$ in bamboo rats, and 62 (40.26%) *E. coli* strains harbored at least two genotypes, which is lower than that reported previously [17, 18]. Furthermore, the rate of phenotypic resistance was less compared to that of genotypic resistance. This difference may be due to the fact that we could not examine the resistance against all β-lactam antibiotics clinically used in China for disease prevention or as a growth promoter. Additionally, the high percentage of genotypic resistance may be due to the irrational use of specific β-lactams or brands used in study farms.

Conclusion

Our study on β-lactam resistance and genotypes provides a guideline for clinical medication and a basis for scientific research regarding resistance-gene transfer between bacteria in bamboo rats.

COMPETING INTERESTS. The authors declare no competing interests.

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