Research Article

Molecular Characterization of Antimicrobial Resistance in *Escherichia coli* from Rabbit Farms in Tai’an, China

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1. Introduction

Antimicrobials, which play a crucial part in animal and human health care, are commonly used to treat bacterial infections and to prevent further spread of the infections within animal groups [1, 2]. *E. coli* are commensal bacteria colonizing the intestinal tract of rabbits and other warm-blooded animal species [3]. They may frequently be exposed to selection pressure caused by antimicrobial treatments and may contribute considerably to the emergence of antimicrobial resistant bacteria and the spread of antimicrobial resistance [4]. Furthermore, antimicrobial resistant bacteria can be transferred to the humans through the food chain, thus affecting human health.

There are many kinds of molecular mechanisms underlying antimicrobial resistance in bacteria. The mechanisms mediated by integrons are an example of the most studied ones. Integrons, one kind of genetic element, are able to capture exogenous genes and disseminate them through plasmids or transposons in both interspecific and intraspecific ways [5].

Genotyping methods of bacteria have been important in surveillance and analysis, and they enable determination of sources of outbreaks. MLST is based on DNA sequencing data, which makes it specific, repeatable, comparable, and suitable for detecting the evolution of the strains and genetic analysis of microbial populations [6, 7].

Most previous studies were focused on the prevalence of antimicrobial resistance of *E. coli* in the main food animal species such as chicken and pigs, but there is a lack of research on antimicrobial resistance in rabbits. Therefore, in this study, we collected faecal samples from rabbit farms in the Tai’an area and isolated *E. coli* strains and then analyzed their drug resistance characteristics and the genotypes, in order to provide information to determine reasonable use of antibiotics.

1. Introduction

Antimicrobials, which play a crucial part in animal and human health care, are commonly used to treat bacterial infections and to prevent further spread of the infections within animal groups [1, 2]. *E. coli* are commensal bacteria colonizing the intestinal tract of rabbits and other warm-blooded animal species [3]. They may frequently be exposed to selection pressure caused by antimicrobial treatments and may contribute considerably to the emergence of antimicrobial resistant bacteria and the spread of antimicrobial resistance [4]. Furthermore, antimicrobial resistant bacteria can be transferred to the humans through the food chain, thus affecting human health.

There are many kinds of molecular mechanisms underlying antimicrobial resistance in bacteria. The mechanisms mediated by integrons are an example of the most studied ones. Integrons, one kind of genetic element, are able to capture exogenous genes and disseminate them through plasmids or transposons in both interspecific and intraspecific ways [5].
and Dongping regions in Tai’an, China. The samples were independently collected from individual animals and the farms were chosen based on their scale (the breeding stock was >1500 heads). According to current statistics, there are about 15 relatively large rabbit farms in Tai’an, and each farm presumably produces >4000 heads annually. Because the sampling process did not harm the animals, ethical approval was not required for the study.

2.2. Samples and E. coli Isolation. Isolation and identification of E. coli were performed as described previously [8], with some modifications. Briefly, about 0.5 g faecal samples were added to a tube containing 5 mL of LB (Luria-Bertani) medium and cultured at 37°C for 12h. The bacteria solution was then streaked onto eosin-methylene blue agar (EMB; Hope, Qingdao, China) plates. After incubation at 37°C for 18~24 h, typical colonies on the plate were streaked on MacConkey’s agar (MAC; Hope, Qingdao, China) plates. Positive colonies were chosen for further biochemical identification using the API 20E system (Sysmex bioMérieux, Tokyo, Japan).

2.3. Antimicrobial Susceptibility Testing. Antimicrobial susceptibility was tested by the Minimal Inhibition Concentration (MIC) method. Bacteria were cultured at 37°C in the LB broth medium for 6 h. The concentration of E. coli was adjusted to 1.5 × 10⁸ colony forming unit (CFU)/mL in sterile saline. The susceptibility of the E. coli isolates was tested to 14 commonly used antimicrobial agents, including ciprofloxacin (CIP), chloramphenicol (C), nalidixic acid (NA), amoxicillin/clavulanic acid (AML), tobramycin (TB), ceftazidime (CAZ), ceftriaxone (CRO), gentamicin (GEN), sulfamethoxazole/trimethoprim (SXT), imipenem (IMP), tetracycline (TET), ampicillin (AMP), cefoxitin (FOX), and amikacin (AMK). The results were interpreted based on the Clinical and Laboratory Standards Institute (CLSI) standard guidelines [9]. In this study, the E. coli strain ATCC25922 was used as the quality control strain. E. coli isolates that were resistant to more than three classes of antimicrobials were defined as multidrug-resistant (MDR) isolates.

2.4. Detection of Resistance Genes. The E. coli DNA was extracted using Bacterial Genomic DNA Extraction Kit (Tiangen, Beijing, China) and then the resistance genes associated with beta-lactams (blaTEM, blaSHV, blaOXA, blABAPE, and blaCTXM), the quinolone-resistance genes (qnrA, qnrB, qnrS, aac(6)-Ib-cr, and qepA), the aminoglycosides-resistance genes (aac(3)-I, aac(3)-II, aac(3)-III, aac(3)-IV, ant(2), and aac(6)-IIb), the tetracycline-resistance genes (tetA and tetB), the sulfonamides-resistance genes (sul1, sul2, and sul3), and the chloramphenicol-resistance genes (cmIA and flor) were amplified by PCR, using the previously reported [10–12] primers and conditions, and the gene sequences were listed in Table 1. In addition, the PCR procedure was repeated twice for all the isolates, and PCR products were analyzed by 1.5% agarose gel electrophoresis at 100 V for 1 h. Photos were taken with a gel imaging system (Tanon-2500, Shanghai, China).

2.5. Detection of Integrons. The universal primers for the amplification of Class 1 integron gene cassette genes were detected by PCR. The primers of cassette FP and cassette RP were designed according to reference: 5′-TCATGGCTTTAGTGACTGT-3′ and 5′-GTAAGGCTTTATTATGCA-3′. The amplification consisted of an initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 60 s, annealing at 56°C for 55 s, and extension at 68°C for 6 min. A final extension for 10 min at 72°C was also applied. PCR products were purified and sequenced for the further analysis [13].

2.6. MLST. According to http://bigdb Pasteur.fr/ecoli/primers_used.html, 8 pairs of primers for housekeeping genes (dinB, icdA, pabB, polB, putP, trpA, trpB, and uidA) were designed and then used for PCR. The products of PCR amplification were sequenced by Shanghai Sangon Biotech Co., Ltd. and the results were amended using the Chromas and DNA Star software and then submitted to the Pasteur online database (http://bigdb Pasteur.fr/perl/bigdb/bigdb.pl?db=pubmlst_ecoli_seqdef_public&sequenceQuery) for processing. Then the allele number of each housekeeping gene was obtained and the sequence type (ST type) of each strain was acquired.

3. Results

3.1. Isolation of E. coli. A total of 55 E. coli strains were isolated from 60 samples: 18 strains were from Ningyang, 20 strains were from Xintai, and 17 strains were from Dongping (Table 3).

3.2. Antimicrobial Susceptibility Test. From Table 2, all the strains were sensitive to ceftazidime, ceftriaxone, imipenem, and amikacin. The highest resistance rate was shown against tetracycline (46/55, 82%) and ampicillin (36/55, 65.5%). The two most common antimicrobial resistance profiles of the isolates were AMP-NA-TET (n = 10, 18.1%) and AMP-TET (n = 8, 14.5%). In addition, 6 strains were sensitive to all the 14 kinds of antibiotics, while there were 50.9% (28/55) multidrug-resistant strains (Table 3).

3.3. Detection of Resistance Genes. Two kinds of beta-lactamase genes, blaTEM and blaCTXM, were the most frequently detected genes at 98.2% and 94.5% respectively, followed by sul2, a kind of sulfa drug-resistant gene, with the detection rate of 58.18%. 5 (9.1%) strains had tetB, a tetracycline-resistance gene. 3 strains (5.5%) had shown resistance to qnrS and 4 strains (7.5%) to aac(6)-Ib-cr, which were quinolone-resistance genes (Table 3).

3.4. Detection of Integrons. Class I integrons were detected in 17 E. coli isolates out of 55 strains, as the detection rate was 30.9%. Seven kinds of drug-resistance gene cassette were found in those Class I integrons as follows: dfrA17 + aadA5, dfrA1 + catB3 + aacA4, aadA2 + LinF, dfrA1 + aadA1, aadA22, dfrA12 + orfF + aadA2, and aadA16 + dfrA27 + arr-3. The most prevalent gene cassettes were dfrA1 + catB3 + aacA4 and aadA2 + LinF (Table 3).

3.5. MLST. 13 different kinds of STs were identified among all the 55 strains, including 3 STs (ST302, ST468, and ST370)
Table 1: Primers for PCR in this study.

| Primer name | Sequence (5’ → 3’) | Reference |
|-------------|--------------------|-----------|
| blaTEM      | F: ATTCTTTGAAGACGAAAGGCC | [10] |
|             | R: ACGCTTCAGTGGAGCAGGAAAAC | |
| blaSHV      | F: CACTCAAGGATGTATTTGTG | [10] |
|             | R: TTAGGGTTCGAGTGCTCG | |
| blaOXA      | F: ACAAAATACATATCAACTTGCC | [10] |
|             | R: AGTGTGTTTGAATGGTGATC | |
| blaPSE      | F: TTTGAGTCTCGCTGAAAGTCG | [10] |
|             | R: TACTCCGAGCACAACATCG | |
| blaCTX-M    | F: CGGTGCGAGATGTCGAGCAG | [11] |
|             | R: ACCGCGATATCGTTG | |
| qnrA        | F: ATTCTTCAAGCAGGATTGG | [12] |
|             | R: TGCCAGCAAGATCTCGAC | |
| qnrB        | F: CGACCTKACGGGCGACTGAAT | [12] |
|             | R: GAGCAACGAYCGCCTGTAGYTG | |
| qnrS        | F: ATGGAAACCTACAATACAC | [12] |
|             | R: AAAACACCTCGACTTAGT | |
| acc(6’)-Ib-cr | F: TTGGATGCTCCTATGAGTGGCTTA | [12] |
|             | R: CTGGAATGCTGCGGTGTTT | |
| qepA        | F: GCAGGTCACGGCAGGTTAG | [12] |
|             | R: CTTCCTGCAGGAGCTAGTG | |
| aac(3)-I    | F: ACCTACTCCAAACATACAGC | [10] |
|             | R: ATATAGCTCCTAGCGGC | |
| aac(3)-II   | F: ACTGTGATGGGATACCCGTC | [10] |
|             | R: CTCCGTCAGCGTTTACGCTA | |
| aac(3)-III  | F: CACAAGAACGATGCTCCGTA | [10] |
|             | R: AACGAGTTAGCAGCACGCTTAC | |
| aac(3)-IV   | F: CTCCGATGCTGCGGTGTTT | [10] |
|             | R: TCATTCTGCTTGCTGCTCAT | |
| ant(2”°)    | F: ATGTTCAGCAGCCAGGATCAGG | [10] |
|             | R: CCGTGCATGATCTATGCTG | |
| acc(6’)-Ib  | F: TTGGATGCTCCTATGAGTGGCTTA | [10] |
|             | R: CTGGAATGCTGCGGTGTTT | |
| tetA        | F: GCCGCTTTCCTTGGTTCT | [10] |
|             | R: CCACCGGTTCAAGGTTGA | |
| tetB        | F: CATTATAGGCGGCACTGCTG | [10] |
|             | R: TGAAGGTCAGTACGAGG | |
| sul1        | F: TGGTAGCAGGATGGCGATTC | [10] |
|             | R: GCGAGGGTTTCCGAGAAATG | |
| sul2        | F: CGGCATCGTCAACATAACC | [10] |
|             | R: GTGTGCGGAGTGAAGTCAG | |
| sul3        | F: CATCCTAGAAGGACTGATCTCG | [10] |
|             | R: CATCAGCAGTAAACCTAGGGGCTTGG | |
| cmlA        | F: TGTCATTACGCCATCTCG | [10] |
|             | R: ATCAGGCACTCCATATCCCAT | |
| flor        | F: CTTGAGGGTGTGTCAGTACTAC | [10] |
|             | R: GCTCCGAGAATGCTGACTAT | |
from samples derived from Ningyang, 6 STs (ST87, ST302, ST314, ST370, ST468, and ST636) from samples derived from Xintai, and 8 STs (ST2, ST24, ST88, ST353, ST370, ST461, ST731, and ST739) from samples derived from Dongping. It was shown that ST302 (22/55, 40.0%) was the most prevalent type, followed by ST370 (12/55, 21.8%) (Table 3).

### 4. Discussion

Colibacillosis of rabbits, a common disease in rabbits breeding, had become one of the most infectious diseases that endanger the rabbit breeding industry [14, 15]. In this study, the highest resistance rates of 55 E. coli strains were against tetracycline and ampicillin (78.2% and 65.5%, resp.), higher than those reported from wild rabbits in Portugal, in which the tetracycline and ampicillin resistance rates were both 11.4% [16]. This coincided with the fact that tetracycline and ampicillin have been widely used to control and prevent rabbit disease in the Tai’an area. In this study, all the strains were sensitive to cefazidime, ceftriaxone, imipenem, and amikacin; this may be due to the less frequent use of these antibiotics in rabbit farms. The occurrence of MDR strains is a potential threat to the public health. Our data showed that the proportion of MDR strains among all the E. coli strains was 50.9%, which was lower than that observed from wild rabbits (71.4%) [16], which may be due to the fact that wild rabbits and hares are commonly found in areas inhabited by human beings and livestock and consequently may share common sources of exposure to antibiotics.

**bla**\textsubscript{TEM}, one kind of β-lactamase genes, is the most common mechanism of ampicillin resistance in E. coli, and it was previously shown in ampicillin-resistant E. coli isolates from foods, humans, and healthy animals in Spain [17]. In this study, 98.2% of E. coli isolates carried **bla**\textsubscript{TEM}, whereas only 61.8% were resistant to ampicillin; this may be associated with the expression status of **bla**\textsubscript{TEM} genes and is needed to be further studied. Extended-spectrum cephalosporins are critically important antibiotics in human medicine [18], which are also used to treat food-producing animals. The acquisition of extended-spectrum cephalosporins resistance was mainly mediated by extended-spectrum β-lactamases in E. coli, while the predominant ones were the CTX-M [19]. In this study, 94.5% of E. coli isolates carried **bla**\textsubscript{CTX-M\textsubscript{V}}, whereas no strains were resistant to cephalosporins; the reason may be that the resistance genes had not yet led to the resistance phenotype. Phenotype could be controlled and verified with the MIC methods. In addition, the quinolone-resistance genes, *qnrS* (5.5%) and *aac(6)-Ib-cr* (7.5%), were much less frequent than the report in Sichuan, where *aac(6)-Ib-cr* (80.4%) and *qnrS* (59.8%) were observed [20]; the difference may be the result of the less frequent use of fluoroquinolone in the Tai’an area, while the detection of the quinolone-resistance genes in this study may come from other sources, such as the contaminated water and food. It has been reported that *tetA* and *tetB* are associated with tetracycline resistance from human and animal sources [21], but our results differed in that tetracycline-resistant E. coli isolates in our rabbit were not all carrying *tetA* or *tetB*; this may be related to regional differences and different breeding conditions. With regard to chloramphenicol-resistant strains, most of them were carrying *flor* genes; however, *cmlA* genes were not very common among chloramphenicol-resistant strains, which was different from other reports, where the *flor* and *cmlA* genes occurred with the same percentage [10]; this difference may be the result of the fact that only partial genes were expressed. The *sul2* and/or *sul3* genes were detected in all of the sulfamethoxazole/trimethoprim-resistant E. coli isolates obtained from rabbits.

In this study, Class I integrons were detected in 17 E. coli isolates out of 55 strains (30.91%), which was similar to the percentage (31.5%) of a previous study conducted by Hai et al. [22] but is lower than that of a previous study observed by Dotto et al. (2014) who detected 61.1% of E. coli strains isolated from domestic and wild Lagomorphs in northern Italy between 2006 and 2008 carrying Class I integrons [23]. Of note, Class I integrons are often associated with MDR
| Number | Location | ST       | Resistance phenotype | Integrons/resistance         |
|--------|----------|----------|----------------------|------------------------------|
| (1)    | Ningyang| ST302    | AMP-NA-TET           | blaCTX,M, blaTEM, sul2       |
| (2)    | Ningyang| ST302    | AMP-TET              | blaCTX,M, blaTEM, sul2       |
| (3)    | Ningyang| ST302    | AMP-NA-TET           | Class 1 (dfrA17 + aadA5), blaCTX,M, blaTEM, sul2 |
| (4)    | Ningyang| ST302    | AMP-TET              | blaCTX,M, blaTEM, sul2       |
| (5)    | Ningyang| ST302    | AMP-C-NA-SXT-TET     | Class 1 (dfrA17 + aadA5), blaCTX,M, blaTEM, flor |
| (6)    | Ningyang| ST468    | AMP-C-NA-SXT-TET     | blaCTX,M, blaTEM, sul2       |
| (7)    | Ningyang| ST468    | AMP-C-NA-SXT-TET     | Class 1 (dfrA17 + aadA5), blaCTX,M, blaTEM, sul2 |
| (8)    | Ningyang| ST302    | AMP-TET              | blaCTX,M, blaTEM, sul2       |
| (9)    | Ningyang| ST302    | AMP-TET              | blaCTX,M, blaTEM, sul2       |
| (10)   | Ningyang| ST468    | AMP-C-NA-SXT-TET     | Class 1 (dfrA17 + aadA5), blaCTX,M, blaTEM, sul2 |
| (11)   | Ningyang| ST302    | AMP-TET              | blaCTX,M, blaTEM, sul2       |
| (12)   | Ningyang| ST302    | AMP-NA-TET           | blaCTX,M, blaTEM, sul1       |
| (13)   | Ningyang| ST302    | AMP-NA-TET           | blaCTX,M, blaTEM, sul2       |
| (14)   | Ningyang| ST370    | AMP-NA-TET           | blaCTX,M, blaTEM, sul2       |
| (15)   | Ningyang| ST370    | AMP-NA-TET           | blaCTX,M, blaTEM, sul2       |
| (16)   | Ningyang| ST370    | AMP-NA-TET           | blaCTX,M, blaTEM, sul2       |
| (17)   | Ningyang| ST370    | AMP-NA-TET           | blaCTX,M, blaTEM, sul2       |
| (18)   | Ningyang| ST370    | AMP-NA-TET           | blaCTX,M, blaTEM, sul2       |
| (19)   | Xintai   | ST370    | SXT                  | Class 1 (dfrA17 + aadA5), blaCTX,M, blaTEM, qnrS, sul1 |
| (20)   | Xintai   | ST87     | GEN-SXT              | blaCTX,M, blaTEM, sul1       |
| (21)   | Xintai   | ST302    | AMP-GEN-SXT-TET      | blaCTX,M, blaTEM, sul1       |
| (22)   | Xintai   | ST302    | AMP-NA-TET           | blaCTX,M, blaTEM, sul2       |
| (23)   | Xintai   | ST370    | AMP-NA-TET           | blaCTX,M, blaTEM, sul2       |
| (24)   | Xintai   | ST370    | AMP-NA-TET           | blaCTX,M, blaTEM, sul2       |
| (25)   | Xintai   | ST314    | NA-TET               | Class 1 (dfrA1 + catB3 + aacA4), blaCTX,M, blaTEM, sul1, sul2 |
| (26)   | Xintai   | ST302    | AMP-C-NA-SXT-TET     | blaCTX,M, blaTEM, sul1       |
| (27)   | Xintai   | ST302    | AMP-FOX-NA-TET       | blaCTX,M, blaTEM, sul1       |
| (28)   | Xintai   | ST302    | TET                  | blaCTX,M, blaTEM, sul2       |
| (29)   | Xintai   | ST302    | AMP-NA-SXT           | Class 1 (dfrA16 + dfrA27 + arr-3), acc(6')-Ib-cr, blaCTX,M, blaTEM, sul2 |
| (30)   | Xintai   | ST636    | AMP-NA-TET           | blaCTX,M, blaTEM, sul1       |
| (31)   | Xintai   | ST370    | AMP-NA-TET           | blaCTX,M, blaTEM, sul2       |
| (32)   | Xintai   | ST468    | AMP-NA-TET           | blaCTX,M, blaTEM, sul2       |
| (33)   | Xintai   | ST468    | AMP-C-NA-SXT         | Class 1 (dfrA1 + catB3 + aacA4), blaCTX,M, blaTEM, sul1 |
| (34)   | Xintai   | ST302    | AMP-NA-TET           | blaCTX,M, blaTEM, sul2       |
| (35)   | Xintai   | ST302    | AMP-NA-TET           | blaCTX,M, blaTEM, sul2       |
| (36)   | Xintai   | ST302    | AMP-NA-TET           | blaCTX,M, blaTEM, sul2       |
| (37)   | Xintai   | ST302    | AMP -TET             | Class 1 (dfrA1 + catB3 + aacA4), blaCTX,M, blaTEM, sul2 |
| (38)   | Xintai   | ST370    | NA-TET               | Class 1 (dfrA1 + catB3 + aacA4), blaCTX,M, blaTEM, sul1 |
| (39)   | Dongping | ST341    | AMP-NA-SXT           | blaTEM, qnrS, sul1           |
| (40)   | Dongping | ST731    | TET                  | blaTEM                        |
| (41)   | Dongping | ST739    | AMP-NA-TET           | acc(6')-Ib-cr, blaCTX,M, blaTEM |
| (42)   | Dongping | ST370    | TET                  | acc(6')-Ib-cr, blaCTX,M, blaTEM |
| (43)   | Dongping | ST379    | AMP-C-NA-SXT-TET     | acc(6')-Ib-cr, blaCTX,M, blaTEM |
| (44)   | Dongping | ST370    | TET                  | acc(6')-Ib-cr, blaCTX,M, blaTEM |
| (45)   | Dongping | ST88     | AML-AMP-C-ALPHA-NA-SXT-TET | Class 1 (dfrA2 + LinF), blaCTX,M, blaTEM, cmlA, flol, sul2, sul3, tetB |
| (46)   | Dongping | ST370    | TET                  | acc(6')-Ib-cr, blaCTX,M, blaTEM |
| (47)   | Dongping | ST739    | AMP-C-NA-SXT-TET     | acc(6')-Ib-cr, blaCTX,M, blaTEM |
| (48)   | Dongping | ST88     | AMP-C-ALPHA-NA-SXT-TET | blaCTX,M, blaTEM, cmlA, flol, sul2, sul3, tetB |
| (49)   | Dongping | ST88     | AMP-C-ALPHA-NA-SXT-TET | blaCTX,M, blaTEM, cmlA, flol, sul2, sul3, tetB |
| (50)   | Dongping | ST88     | AMP-C-ALPHA-NA-SXT-TET | Class 1 (dfrA2 + LinF), blaCTX,M, blaTEM, cmlA, flol, sul2, sul3, tetB |
| (51)   | Dongping | ST353    | C-TET                | Class 1 (dfrA22), blaTEM, flol, qnrS, sul2 |
| (52)   | Dongping | ST370    | NA-TET               | Class 1 (dfrA12 + orfF + addA2), blaCTX,M, sul1 |
Table 3: Continued.

| Number | Location | ST   | Resistance phenotype          | Integrons/resistance                                      |
|--------|----------|------|-------------------------------|-----------------------------------------------------------|
| (53)   | Dongping | ST88 | C-CIP-NA-TET                  | Class 1 (aadA2 + LinF), blaCTX-M, blaTEM, flor, sul2, sul3, tetB |
| (54)   | Dongping | ST24 | AML-AMP-TET                   | Class 1 (aadA22), blaCTX-M, blaTEM, flor, sul1           |
| (55)   | Dongping | ST88 | AMP-C-CIP-GEN-NA-SXT-TB-TET   | Class 1 (aadA2 + LinF), blaCTX-M, blaTEM, cmlA, flor, sul2, sul3, tetB |

E. coli isolates, consistent with the result of the present study. In this study, the highest isolation rate of 40% (22/55) was found for ST302 which had not yet been reported related to infections. It was different from that of previous studies which found that ST40 is the most common ST from rabbits in Italy [23].

5. Conclusions

Our findings exhibit the molecular characterization of antimicrobial resistance in Escherichia coli from rabbit farms in Tai’an, China. The rabbits in farms are contaminated with MDR E. coli, which may originate from farms further up the food chain or from horizontal contamination. The high detection rates of MDR E. coli, Class I integrons, and antibiotic-resistance genes suggested that measures should be taken to reduce the harm to public health, such as the reasonable use of antimicrobials in animal husbandry and culling the reservoirs of pathogens from the slaughter process.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

Xiaonan Zhao and Jie Yang have contributed equally to this work.

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