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*Original Article*

# Characterization and horizontal transfer of class 1 integrons in *Escherichia coli* isolates from cooked meat products

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## Abstract

Introduction: *Escherichia coli* is a commensal bacterium in humans, animals, and the environment that is one of the microorganisms commonly resistant to antimicrobials. Cooked meat products, which are popular in China, are easily contaminated by *E. coli* during processing and storage.

Methodology: In this study, a total of 75 *E. coli* isolates from cooked meat products in Henan province, China, were assayed for the presence of and horizontal transfer of class 1 integrons.

Results: Class 1 integrons were detected in 11 (14.7%) of these isolates, and contained four groups of resistance gene cassettes, including *dfrA17-aadA5*, *dfrA1-aadA1*, *dfrA12-orfF-aadA2*, and an uncommon array of *aacA4-catB8-aadA1*. The transfer frequency of selected integron-positve donors ranged from $10^{-6}$ to $10^{-4}$ transconjugants per recipient cell, and the integron-containing DNA from the donors could be transferred to *E. coli* J53Az$^{r}$ with the transformation frequency of $10^{-7}$ to $10^{-5}$.

Conclusions: Class 1 integrons could be transferred to recipient *E. coli* J53 by conjugation and natural transformation. These findings suggest the role of commensal *E. coli* isolates from cooked meats as an important reservoir for integrons and the possible transfer of antimicrobial resistance genes to humans via the food chain.

**Key words:** *Escherichia coli*; antimicrobial resistance; integron; natural transformation.

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## Introduction

*Escherichia coli* is a commensal bacterium in humans and animals, and can be easily disseminated in different ecosystems through food and water [1]. Due to its ubiquity in humans and animals and its role as a pathogenic and commensal organism, *E. coli* has become one of the microorganisms that are commonly resistant to antimicrobials. It is possible that antimicrobial-resistant isolates of *E. coli* can be transferred to human through the food chain, and their resistance genes can be transferred to other pathogens, which represents a potential risk to public health [1,2].

Cooked meat products belong to ready-to-eat foods that are consumed without further cooking. Such meat products, which are popular in China, are easily contaminated by organisms during processing and storage [3]. In China, data on the distribution and characterization of class 1 integron-carrying *E. coli* from cooked meat products are scarce and fragmented [4]. In previous studies, natural transformation of *E. coli* can be implemented by various artificial transformation methods [5-7]. Recently, a transformation system for *E. coli* that is completely different from the traditional methods has been reported [8]. In this system, non-physiological factors (*e.g.*, a high $Ca^{2+}$ concentration, incubation at a low temperature, a temperature shift, and an electronic shock in electroporation) are not necessary. Thus, the purpose of this study was to investigate the presence of class 1 integrons in meat-borne *E. coli* and the horizontal transfer of class 1 integrons by conjugation and natural transformation using the transformation system mentioned above.

## Methodology

*E. coli isolates*

A total of 75 *E. coli* isolates were recovered from 620 cooked meat products between October 2009 and

May 2011 in Henan province, originating from roasted meats (n = 23), pot-stewed meats (n = 38), sausages (n = 5), and smoked meats (n = 9). Samples were purchased from supermarkets, farmers' markets, cooked meat shops, and street food vendors. The antimicrobial resistance profiles of these isolates have been reported previously [9]. In brief, they are frequently resistant to tetracycline (56.0%), trimethoprim/sulfamethoxazole (41.3%), streptomycin (29.3%), ampicillin (26.7%), and nalidixic acid (14.7%).

*Detection and characterization of integrons*

The presence of the class 1 integron was detected by polymerase chain reaction (PCR) targeting the class 1 integrase gene using previously described primers (Table 1). Gene cassettes within the variable region of class 1 integron were then amplified as in the described method (Table 1). The PCR products were cloned into the pGEM-T vector (Promega, Madison, USA) and sequenced at Invitrogen Biotechnology (Shanghai, China). The resulting DNA sequence data were compared to the GenBank database using the BLAST algorithm available at the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov).

*Conjugation experiments*

Seven of the *E. coli* isolates carrying class 1 integrons with resistance gene cassettes were used as donors, and the *E. coli* J53Az$^r$ (resistant to sodium azide) strain served as the recipient. These isolates were selected as representatives of the seven different pulsotypes. Conjugation experiments were performed using the broth mating method as described previously [12]. Donor and recipient cells (ratio, 1:10) were mixed in Luria-Bertani broth (LB; Huankai, Guangzhou, Guangdong, China) and incubated at 37°C overnight. The mating mixture was streaked onto trypticase soy agar (TSA; Huankai) plates containing a combination of sodium azide (100 μg/mL; Sigma-Aldrich, St. Louis, USA) and tetracycline (8 μg/mL) or a combination of sodium azide (100 μg/mL) and streptomycin (50 μg/mL). Plasmids of the donors and transconjugants were extracted using the High Purity Plasmid Miniprep kit (Dongsheng Biotech, Guangzhou, Guangdong, China), according to the manufacturer's instructions. Then the successful transfer of integrons to the recipient was confirmed by PCR using primers shown in Table 1.

*Natural transformation of* E. coli

The plasmids isolated from seven integron-positive strains were subjected to natural transformation with *E. coli* J53Az$^r$ as the recipient followed the previously described protocol [8]. The recipient strain was grown overnight in LB broth (to reach a density of $10^8$～$10^9$ colony-forming units [CFU]/mL). The culture was inoculated at 1:100 into 5 mL of fresh LB broth and then cultured with shaking at 37°C. When culture growth reached the stationary phase, 100 μL of the culture was inoculated into the fresh tube and incubated for 12 hours at 37°C without shaking. This step was called static culture of stationary phase cells, and was important for transformation efficiency. Then, four micrograms of plasmid DNA was added to each tube, mixed gently, and then spread on LB agar plates supplemented with corresponding antimicrobials. It was concluded that transformation occurred on LB agar plates, because many scholars had found transformation of *E. coli* was easier on solid media than in liquid [6,13]. The transfer of integron was confirmed by PCR amplification and DNA sequence analysis. Transformation frequency was determined as the ratio of the number of transformants/mL to the total CFU/mL of the recipient.

*Pulsed-field gel electrophoresis (PFGE)*

PFGE was performed according to the PulseNet standardized protocol [14]. Briefly, agarose-embedded DNA was digested with 20 U of *Xba*I (CHIMERx, Madison, USA), using *Salmonella* Braenderup H9812 as a reference strain. The restriction fragments were separated using the CHEF MAPPER apparatus (Bio-Rad, Hercules, USA) at 6 V/cm with switch time

**Table 1.** Primers used for polymerase chain reaction amplification in this study

| Target gene | Primer | Sequence (5'-3') | Size (bp) | Reference |
|---|---|---|---|---|
| *intI1* | intI1-F | ACGAGCGCAAGGTTTCGGT | 565 | 10 |
| | intI1-R | GAAAGGTCTGGTCATACATG | | |
| Variable region | in-F | GGCATACAAGCAGCAAGC | Variable | 11 |
| | in-R | AAGCAGACTTGACCTGAT | | |

ranging from 2.2 to 54.2 seconds. The gel was stained in ethidium bromide solution and photographed using a Bio-Rad Gel Doc. PFGE patterns were compared using Quantity One software (Bio-Rad).

## Results

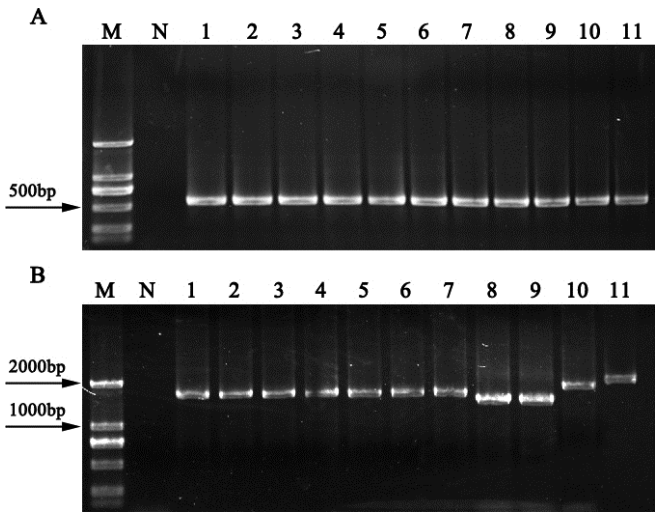
*Distribution of class 1 integrons*

In this study, the 75 *E. coli* isolates were subjected to PCR screening for integrase gene, and the 565-bp corresponding amplicon was detected in 11 isolates, suggesting the presence of class 1 integrase gene (Figure 1A). The *intI1*-positive isolates contained four groups of resistance gene cassettes, consisting of *dfrA17-aadA5* (1,664 bp, n = 7), *dfrA1-aadA1* (1,586 bp, n = 2), *dfrA12-orfF-aadA2* (1,913 bp, n = 1), and *aacA4-catB8-aadA1* (2,184 bp, n = 1) (Figure 1B and Table 2).

*Conjugation experiments*

Transfer of conjugative plasmids is known to be the most common mechanism for genetic exchange between bacteria, as plasmid conjugation can occur at high frequency and is able to transfer resistance genes. In this study, seven of the *E. coli* isolates were selected for conjugation experiments. The seven integron-positve donors produced transconjugants successfully. PCR methods confirmed that the transconjugants harbored the same class 1 integrons as their donors. The transfer frequency of these isolates ranged from $10^{-6}$ to $10^{-4}$ transconjugants per recipient cell (Table 3). Therefore, it was speculated that the class 1 integrons were located on conjugative plasmids among these isolates.

**Figure 1.** PCR-based analysis of *Escherichia coli* isolates carrying class 1 integrons. (A) PCR amplification of the class 1 integrase gene (*intI1*). Lanes: N, E25 (negative control); 1, E10; 2, E42; 3, E33; 4, E128; 5, E259; 6, E215; 7, E332; 8, E15; 9, E287; 10, E231; 11, E321. (B) PCR amplification of variable region of class 1 integron. Lanes: N, E25 (negative control); 1, E33; 2, E128; 3, E259; 4, E215; 5, E332; 6, E15; 7, E287; 8, E10; 9, E42; 10, E231; 11, E321.




*Natural transformation*

In this study, natural transformation of *E. coli* isolates was done using the transformation system described by Sun *et al*. [8]. The results demonstrated that the integron-containing DNA from the seven donors could be transferred to *E. coli* J53Az$^r$ in this transformation system with the transformation frequency of $10^{-7}$ to $10^{-5}$ (Table 3). The PCR amplification confirmed the presence of the *intI1* gene and the gene cassette in the transformants.

**Table 2.** Characteristics of *Escherichia coli* isolates carrying class 1 integrons

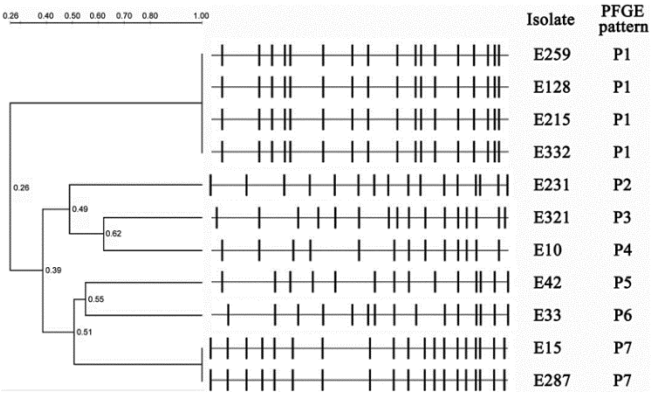
| Strain | Antimicrobial resistance profile | Class 1 integrons | | PFGE pattern |
|---|---|---|---|---|
| | | Size(bp) | Gene cassette | |
| E10 | TET,SXT,STR,AMP,CAZ,GEN | 1,586 | *dfrA1-aadA1* | P4 |
| E42 | TET,SXT,STR,AMP,CHL,GEN | 1,586 | *dfrA1-aadA1* | P5 |
| E33 | TET,SXT,STR,AMP | 1,664 | *dfrA17-aadA5* | P6 |
| E128 | TET,SXT,STR,NAL,CAZ,GEN,FEP | 1,664 | *dfrA17-aadA5* | P1 |
| E259 | TET,SXT,AMP,NAL,CAZ,CHL,CFP,CIP | 1,664 | *dfrA17-aadA5* | P1 |
| E215 | TET,SXT,STR,NAL,CAZ | 1,664 | *dfrA17-aadA5* | P1 |
| E332 | SXT,STR,NAL,CFP | 1,664 | *dfrA17-aadA5* | P1 |
| E15 | SXT,STR,CFP | 1,664 | *dfrA17-aadA5* | P7 |
| E287 | SXT,STR,CFP | 1,664 | *dfrA17-aadA5* | P7 |
| E231 | TET,SXT,STR,NAL,CIP | 1,913 | *dfrA12-orfF-aadA2* | P2 |
| E321 | STR,AMP,CHL,GEN | 2,184 | *aacA4-catB8-aadA1* | P3 |

TET: tetracycline; SXT: trimethoprim/sulfamethoxazole; STR: streptomycin; AMP: ampicillin; CAZ: ceftazidime; GEN: gentamycin; CHL: chloramphenicol; NAL: nalidixic acid; FEP: cefepime; CFP: cefoperazone; CIP: ciprofloxacin.

*PFGE*

PFGE analysis revealed 7 different pulsotypes among the 11 integron-positive isolates (Figure 2). There was no complete correlation between genotypes and specific integron-associated phenotypes. For example, some isolates that carried the same gene cassettes in their integrons had different PFGE patterns. On the contrary, some isolates belonging to the same PFGE patterns possessed different gene cassettes.

**Figure 2.** Dendrogram of PFGE (*Xba*I) profiles of 11 *Escherichia coli* isolates with integrons




## Discussion

In the present study, *dfrA17-aadA5* was the most common gene cassette array, which is similar to other reports of *E. coli* isolates from food, humans, and the environment in China [4,15,16]. However, the distribution of gene cassettes in our study differed from that found in food samples in other countries such as Tunisia and Norway, where *dfrA1-aadA1* was the dominant array [17,18]. The cassette array *aacA4-catB8-aadA1*, encoding tobramycin, chloramphenicol, and streptomycin resistance, respectively, has been found frequently in *Acinetobacter* isolates in many previous studies [19,20,21]. To our knowledge, *aacA4-catB8-aadA1* was not common among *E. coli* isolates [22,23]. However, this array was recently detected in clinical isolate of *E. coli* in Guangzhou, China [22]. The presence of this gene cassette in *E. coli* from cooked meat products in our study indicates that class 1 integrons may play an important role in the horizontal dissemination of antimicrobial resistance in the different bacterial species and the same species from different isolation sources.

All *intI1*-positive isolates showed resistance to three or more classes of antimicrobials. This high frequency of multidrug resistance among *intI1*-positive isolates supports the hypothesis of an association between the presence of class 1 integrons and emerging multidrug resistance in *E. coli* [4,16].

In bacteria, horizontal gene transfer is widely recognized as the mechanism responsible for the widespread distribution of antimicrobial resistance genes, facilitating bacterial adaption and evolution [24]. Resistance determinants are readily acquired and disseminated within and among bacterial populations by conjugation, transduction, and transformation.

Many previous studies have reported that most antimicrobial resistance determinants and class 1 integrons in *E. coli* were encoded in transferable plasmids, which might be transferred via conjugation [12,25]. The transfer of resistant genes from commensal bacteria to pathogens in the human intestine has been found, potentially resulting in food poisoning that is more difficult to treat with conventional antimicrobial agents [26].

Natural transformation is characterized by the uptake of free DNA by a recipient bacterium, its chromosomal integration or extra-chromosomal stabilization, and its expression, which leads to a new phenotype [27]. So far, more than 60 bacterial species, distributed through all taxonomic groups, are known to be naturally transformable [28]. *E. coli* is not considered to be competent for natural transformation, although various artificial transformation methods, such as the addition of $Ca^{2+}$, low temperature, and a temperature shift, have been developed [5-7]. In this

**Table 3.** Frequencies of conjugation and transformation for the selected isolates

| Donor strain | Conjugation frequency | Transformation frequency |
|---|---|---|
| E10 | $3.4\times10^{-5}$ | $7.7\times10^{-6}$ |
| E42 | $4.7\times10^{-5}$ | $4.2\times10^{-7}$ |
| E33 | $3.6\times10^{-4}$ | $5.0\times10^{-7}$ |
| E259 | $2.4\times10^{-6}$ | $1.5\times10^{-6}$ |
| E15 | $4.0\times10^{-4}$ | $2.7\times10^{-5}$ |
| E231 | $6.1\times10^{-6}$ | $9.2\times10^{-6}$ |
| E321 | $6.0\times10^{-6}$ | $8.1\times10^{-7}$ |

study, we performed natural transformation of *E. coli* isolates using a transformation system described by Sun *et al*., in which neither a non-physiological concentration of $Ca^{2+}$ and temperature shifts nor electronic shocks were required. It was proposed that natural competence induction of *E. coli* in this system required two important steps: early competence induction during static culture and late competence induction on plates. Following the simple protocol of this transformation system, the integron-containing DNA from the seven donors could be transferred to *E. coli* J53Az$^r$ in our study. Our results showed that the transformation frequency of *E. coli* J53Az$^r$ using this system ranged from $10^{-7}$ to $10^{-5}$, similar to that with the same system in previous studies, indicating that this transformation system was effective for the natural transformation of *E. coli*. The finding that class 1 integron-encoding plasmids of foodborne *Salmonella* isolates could be transmitted to the oral residential bacterium *Streptococcus mutans* by natural gene transformation was reported in previous study, suggesting that the interspecies acquisition of integrons might be accelerate the spread of antimicrobial resistance [29]. Some researchers consider that *E. coli* has the capacity for natural transformation without the transformation being detectable under laboratory conditions that are limited in sensitivity, time, and by environmental variables [30]. Our data support the idea that *E. coli* is naturally transformable under certain circumstances [27]. In our study, *E. coli* isolates from cooked meat products could acquire the class 1 integrons from the environments via natural transformation, indicating that these isolates may potentially act as reservoirs of resistance genes and play an active role in the transfer of resistance to humans through the food chain.

## Conclusions

Our results demonstrated that cooked meat products might be an important avenue for evolution and dissemination of antimicrobial resistant bacteria. There is a possibility that these resistance genes may transfer to humans via the food chain; therefore, monitoring the occurrence of antimicrobial resistance among bacteria from food in China is urgently needed for the benefit of public health.

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