

# Role of Antibiotic in Drug Resistance and Integrons Prevalence in *Escherichia coli* Isolated from Human and Animal Specimens

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## Abstract

It is believed that high antibiotic consumption and horizontal transfer of resistance genes are two major causes of antibiotic resistance in bacteria. To confirm or reject this belief, we studied the role of drug administration in antimicrobial resistance (AMR), prevalence of Class I and II integrons and integron-mediated resistance in *Escherichia coli* isolated from human and animal specimens. Recording demographic data, *E. coli* from different specimens including human, chicken, cattle and sheep was isolated followed by phenotypic antibiotic susceptibility testing and detection of Class I and II integrase genes. The correlation between integrons and resistance (P value) was evaluated using SPSS software. According to demographic records, chickens received the highest dose and variation of antibiotics. As expected, the most prevalent MDR strains and integrons were found in chicken strains. Chi square analysis showed a significant correlation between integrons and resistance pattern mostly in *E. coli* strains isolated from chicken rather than other specimens. Our survey confirmed that the use of antibiotics is strongly associated with the prevalence of antimicrobial resistance and integrons in commensal *E. coli*. Such results confirm that high doses of antibiotics and selection pressure may remove susceptible intestinal microorganisms followed by resistant ones.

**Keywords:** Antimicrobial resistance, Cattle, Chicken, *Escherichia coli*, Integrons

## İnsan ve Hayvan Örneklerinden İzole Edilen *Escherichia coli*'nin İlaç Direnci ve İntegronların Yaygınlığında Antibiyotik Rolü

### Özet

Yüksek antibiyotik kullanımı ve dayanıklılık genlerinin yatay transferinin bakterilerde antibiyotik direncinin iki önemli nedeni olduğuna inanılmaktadır. Bu inancı onaylamak veya reddetmek için, insan ve hayvan örneklerinden izole edilen *Escherichia coli*'nin antimikrobiyel direnci (AMR), Sınıf I ve II integronları ve integron-araçlı direnç prevalansında ilaç uygulamasının rolünü araştırdık. Demografik verileri kaydetmek için; fenotipik antibiyotik duyarlılık testi ve Sınıf I ve II integraz genlerinin tespiti sonrası insan, tavuk, sığır ve koyun gibi farklı örneklerden *E. coli* izole edildi. İntegronlar ve direnç (p değeri) arasındaki korelasyon SPSS yazılımı kullanılarak değerlendirildi. Demografik kayıtlara göre, tavuklar en yüksek doz ve değişimli antibiyotik aldı. Beklenildiği gibi, en yaygın MDR suşları ve integronları tavuk suşlarında bulundu. Ki kare analizi, başka örnekler yerine çoğunlukla tavuktan izole edilen *E. coli* suşlarının integronları ve direnç modeli arasında anlamlı bir korelasyon gösterdi. Araştırmamız, antibiyotik kullanımının ortakçı *E. coli*'de antibiyotik direnci ve integronların yaygınlığı ile sıkı ilişkili olduğunu doğruladı. Bu sonuçlar, yüksek doz antibiyotik ve seçim baskısının dirençli olanların ardından duyarlı bağırsak mikroorganizmalarını ortadan kaldırdığını doğrulamaktadır.

**Anahtar sözcükler:** Antimikrobiyel direnç, Sığır, Tavuk, *Escherichia coli*, İntegronlar

## INTRODUCTION

Antibiotics are widely used for therapy and control of bacterial infections as well as to promote growth in animals [1-4]. However, over the past decades a rapid increase in the number of antibiotic-resistant clinical isolates has been observed as well as a low rate of development and

introduction of new antimicrobial agents have been occurred [5-7].

There are three principal types of antibiotic resistance, namely, intrinsic, acquired, and adaptive antibiotic resistance [8]. However, antibiotic maladministration stabilizes resistance, in other words selection pressure mediated by antibiotics can shift normal micro flora to antibiotic-



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resistant microorganisms to increase the resistance gene transferring rate<sup>[5]</sup>.

Followed by taking inadequate antibiotics, the emergence of *E. coli* isolates with multiple antibiotic-resistant phenotypes, involving co-resistance to four or more unrelated families of antibiotics, has been previously reported and it has been suggested that resistance in bacterial populations may spread from one ecosystem to another by lateral gene transfer, specifically integrons<sup>[9,10]</sup>. Integrons are bacterial genetic elements that allow the shuffling of smaller mobile elements called gene cassettes; they have been termed a genetic construction kit for bacteria<sup>[11-13]</sup>. The main components of an integron are an integrase enzyme (*IntI*), a recombination site (*attI*) and a promoter located upstream of the integration site. Integrons are involved in the evolution and spread of antibiotic-resistance genes in enteric bacteria. There are various classes of integrons<sup>[14]</sup>, according to their integrases and associated cassettes<sup>[15,16]</sup>. Since many gene cassettes of integrons contain AMR genes in Gram-negative bacteria, the horizontal transfer of integrons through plasmids and transposons has been found to play an important role in the dissemination of AMR genes and the development of multiresistance<sup>[16]</sup>. As a result of the variation of antibiotics taken by human, chicken, cattle, and sheep, and selection mediated by such drugs, it is probable to observe different patterns of resistance in *E. coli* strains isolated from different specimens.

In fact, in current study the role of drug administration in AMR, prevalence of Class I and II integrons and integron mediated resistance in *E. coli* isolated from human and animal specimens were evaluated.

## MATERIAL and METHODS

### Ethics

Human stool (faeces) samples were collected in accordance with the bioethics organizations in Iran (including The Ministry of Health and Medical Education, Office of Study for Humanistic and Islamic Science on Medicine and Medical Ethics). For human stool samples, IRB approval was obtained from Tehran University of Medical Sciences. For animal samples, Permission was obtained from Alborz University of Veterinary Sciences and Institutional Animal Care and Use Committee (IACUC) approved this specific study. To collect samples, written information about the study was given to the owners of breeding farm and facility and Informed consent was obtained. A questionnaire including sex, age, antibiotic diet and enteric disease was filled and signed by the human participants and consent form was recorded by the authors.

### Samples Collection

This study was performed from August 2015 to October

2015. The authors collected the samples from four sources: Healthy volunteer human not using any antibiotic for 2 weeks before sampling from Amini Medical Laboratory located in Alborz province, large intestine swabs from chicken, cattle, and sheep faeces. Faecal samples were collected from living animal and so no animal was sacrificed. Chicken enteric specimens were obtained from private animal breeding farm Qadir in Karaj city (suburb of Alborz province with geographic coordinate of 35.8840059, 50.9716793), while sheep and cattle enteric samples were collected from private facility Raeesi located in Zavareh city (suburb of Isfahan province with geographic coordinate of 33.449974, 52.490830).

To collect samples, oral and written information about the study was given to each human participant and owners of breeding farm and facility and an informed consent was obtained. A questionnaire about information pertaining to the sex, age, antibiotic diet and enteric disease was filled by the human Participants.

### Bacterial Isolates

To isolate *E. coli*, faecal samples were inoculated to Lauryl Sulphate Tryptose (LST) Broth (Merck, Germany) broth followed by EC broth (Merck, Germany) at 44.5°C and streaked on EMB agar (Merck, Germany). Colonies showing metal sheen were considered as presumptive *E. coli* isolates and underwent IMViC test for final confirmation<sup>[17]</sup>.

### Demographic Data Collection

While collecting faecal samples, the antibiotic consumption of each participant was recorded. According to this data, highly different patterns of antibiotics were used, among which chicken prescribed intensive antibiotic diets were including soltrim (trimetoprima sulfametoxazol), fozbac, tetracycline, doxycycline, chloramphenicol, enrofloxacin, gentamicin, furazolidone and colistin. Tetracycline, trimetoprima and penicillin had been administered for cattle, while sheep were fed with oxytetracycline, chlortetracycline, neomycin sulphate, ceftiofur sodium and spectinomycin. However, human participants included in this study were healthy volunteers who had not taken any antibiotic for at least two weeks before sampling.

### Antimicrobial Susceptibility Testing

Phenotypic antibiotic susceptibility was tested applying Pad tan Teb (Tehran, Iran) disks by Kirby-Bauer disk diffusion method on Mueller-Hinton agar plates according to the guidelines of Clinical and Laboratory Standards Institute<sup>[18]</sup>. A panel of 24 antibiotic discs containing 7 categories listed in Table 3-6 were tested and *E. coli* ATCC 25922 was used as quality control strain. For antimicrobial susceptibility testing, the inoculum of *E. coli* was suspended in the sterile saline solution (0.85% NaCl) with a sterile swab to adjust turbidity to match the 0.5 McFarland standards, and streaked evenly on Mueller-Hinton agar plates. Plates were

incubated inverted at 35°C for 18 h and the inhibition zone diameters were measured [18].

### PCR Assay for Detection of Resistance Genes

To extract the genomic DNA, bacterial cells were centrifuged at 2.500 round per min (rpm) for 15 min. Discarding the supernatant, following manufacturer's protocol (Bioneer's AccuPrep Genomic DNA Extraction Kit), DNA from all isolates was extracted.

To detect Class I and II integrase genes, two sets of primers were designed (listed in Table 1) a new duplex PCR was developed to detect both genes simultaneously.

The gene amplification protocol was performed by ABI verity 96 well thermal cycler in reaction mixture at a final volume of 25 µL consisting of 12.5 µL, 2x CinnaGen PCR master kit containing *Taq* DNA Polymerase (recombinant), PCR buffer, MgCl<sub>2</sub>, dNTPs, in addition to 2 µ DNA template and specific pmols of each set of primers. The temperature profile for integrase genes amplification was as follows: initial denaturation (94°C for 5 min), followed by 30 cycles of denaturation (94°C for 20 sec), annealing (30 s at 60°C), and extension (72°C for 1 min); and then a final extension (72°C for 10 min).

PCR products were evaluated by electrophoresis in 1% agarose gel containing SYBR green and visualized by a Gel DOC™ XR<sup>+</sup> (BIORAD) and analysed by Image Lab™ 4.0 software.

### Statistical Analysis

The SPSS software (version 19) was used for statistical analysis. The association between presence of integrons

and antibiotic resistance was determined by  $\chi^2$  or Fisher's exact test. A *P* value of < 0.05 was considered statistically significant.

## RESULTS

### Specimens

Considering the questionnaires, human taking antibiotics within 2 weeks were excluded; hence 50 specimens (isolates) from 29 male and 21 female, aged 17-43 years participated in the study. In addition to human isolates, 50 faecal samples were collected from each three animal species and thus 200 isolates were included in this study.

### Antimicrobial Resistance

Antibiotic susceptibility results showed no extensively drug-resistant (XDR isolate); however, 186 (93%) isolates were MDR (resistant to at least 1 category) and 14 isolates were susceptible to 1 category. Of the 200 *E. coli* isolates from different specimens, the overall rates of resistance to antimicrobial agents were higher in chicken isolates than in the others since among the isolates, chicken isolates showed the highest resistance to all categories while the number of isolates resistant to 2, 3, 4, 5 and 6 categories were 3, 9, 10, 17 and 11, respectively (Table 2).

Overall results showed that the highest and the lowest resistance to cepheims belongs to cephalothin and ceftazidime while for aminoglycosides, amikacin and tobramycin showed the highest and the lowest resistance, respectively (Table 3, Table 4). Penicillins, were also used in this study among which piperacillin had the best anti-

**Table 1.** Primers used for amplification of Class I and II integrase genes

**Tablo 1.** Sınıf I ve II integraz genlerinin amplifikasyonunda kullanılan primerler

Gene	Sequence (5' → 3')	Product Size (bp)	Annealing (Temperature)
<i>Int 1</i>	Forward: 5'TCTCGGGTAACATCAAGG3' Reverse: 5'GTTCTTCTACGGCAAGGT3'	287	60
<i>Int 2</i>	Forward: 5' CACGGATATGCGACAAAAAGGT 3' Reverse: 5' GTAGCAAACGAGTGACGAAATG 3'	789	60

**Table 2.** Source and number of resistant isolates to antimicrobial categories

**Tablo 2.** Antimikrobiyel kategorilerine dirençli izolatların kaynak ve sayısı

No. of Resistant Categories	No. of Chicken Isolates	No. of Human Isolates	No. of Cattle Isolates	No. of sheep Isolates	Sum
Resistant to 7 categories	0	0	0	0	0
Resistant to 6 categories	11	0	1	1	13
Resistant to 5 categories	17	6	0	0	23
Resistant to 4 categories	10	12	7	4	33
Resistant to 3 categories	9	22	23	13	67
Resistant to 2 categories	3	10	17	20	50
Resistant to 1 category	0	0	2	12	14
Resistant to 0 category	0	0	0	0	0

microbial activity since just one chicken isolate showed resistance (Table 5).

### Detection of Integrons

Of the 200 *E. coli* isolates tested, 55 (27.5%) carried *Int I* and 19 (9.5%) carried *Int II* while 9 (4.5%) isolates carried both classes. Class I integrase gene was detected in 50% (25/50), 38% (19/50), 6% (3/50) and 16% (8/50) while Class II integrase gene was detected in 26% (13/50), 8% (4/50), 0% (0/50), and 4% (2/50) of chicken, human, cattle and sheep isolates, respectively. Among the isolates, 14% (7/50) of chicken and 4% (2/50) of sheep harbored both classes of integrase genes simultaneously.

To analyse the relationship between the prevalence of integrase gene and resistance, the software SPSS 19.0 was used to evaluate the *p* value of Fisher's exact test. In some strains, integron carriage significantly caused correlated resistance including Class I integron and CTX ( $P < 0.001$ ), CRO ( $P < 0.001$ ), SXT ( $P < 0.001$ ), AMC ( $P = 0.001$ ), AMP ( $P = 0.009$ ), S ( $P = 0.009$ ), NOR ( $P = 0.023$ ), NA ( $P = 0.027$ ) and LEV ( $P = 0.05$ ) resistance in chicken originated strains, SXT ( $P < 0.001$ ) in cattle originated strains, SXT ( $P = 0.003$ ) and GM ( $P = 0.02$ ) resistance in sheep originated strains and AN ( $p = 0.016$ ) resistance in human originated strains. However less prevalence of Class II integron caused less correlated antibiotic resistance including, S ( $P < 0.001$ ) resistance in chicken

**Table 3.** Antimicrobial resistance of *E. coli* isolates from human and animals

**Table 3.** İnsan ve hayvanlardan izole edilen *E. coli* antimikrobiyel direnci

Specimen	No. (%) of Isolates Resistant to Antimicrobial Agents						
	Cephems						
	CAZ* (30 µg)	CTX* (30 µg)	CRO* (30 µg)	CT* (30 µg)	CF* (30 µg)	CZ* (30 µg)	CN* (30 µg)
Human	1 (2%)	19 (38%)	5 (10%)	2 (4%)	47 (94%)	5 (10%)	19 (38%)
Chicken	3 (6%)	28 (56%)	3 (6%)	5 (10%)	47 (94%)	5 (10%)	14 (28%)
Cattle	0 (0%)	5 (10%)	0 (0%)	0 (0%)	50 (100%)	0 (0%)	0 (0%)
Sheep	1 (2%)	9 (18%)	0 (0%)	2 (4%)	47 (94%)	2 (4%)	18 (36%)
Total	5 (2.5%)	61 (30.5%)	8 (4%)	9 (4.5%)	191 (95.5%)	12 (6%)	51 (25.5%)

\* CAZ: ceftazidime, CTX: cefotaxime, CRO: Ceftriaxone, CT: ceftizoxime, CF: cephalothin, CZ: cefazolin, CN: cephalixin

**Table 4.** Antimicrobial resistance of *E. coli* isolates from human and animals

**Table 4.** İnsan ve hayvanlardan izole edilen *E. coli* antimikrobiyel direnci

Specimen	No. (%) of Isolates Resistant to Antimicrobial Agents					
	Aminoglycosides					
	S* (10 µg)	TOB* (100 µg)	AN* (30 µg)	K* (30 µg)	N* (30 µg)	GM* (10 µg)
Human	5 (10%)	46 (92%)	0 (0%)	3 (6%)	8 (16%)	1 (2%)
Chicken	7 (14%)	36 (72%)	0 (0%)	8 (16%)	11 (22%)	9 (18%)
Cattle	0 (0%)	41 (82%)	0 (0%)	2 (4%)	0 (0%)	0 (0%)
Sheep	2 (4%)	21 (42%)	0 (0%)	4 (8%)	8 (16%)	1 (2%)
Total	14 (28%)	144 (72%)	0 (0%)	17 (8.5%)	29 (14.5%)	11 (5.5%)

\* S: Streptomycin, TOB: Tobramycin, AN: Amikacin, K: Kanamycin, N: Neomycin, GM: Gentamycin

**Table 5.** Antimicrobial resistance of *E. coli* isolates from human and animals

**Table 5.** İnsan ve hayvanlardan izole edilen *E. coli* antimikrobiyel direnci

Specimen	No. (%) of Isolates Resistant to Antimicrobial Agents			
	Folate Pathway Inhibitors	Penicillins		
	SXT* (25 µg)	AMC* (20/10 µg)	PRL* (100 µg)	AMP* (10 µg)
Human	0 (0%)	16 (32%)	0 (0%)	12 (24%)
Chicken	5 (10%)	34 (68%)	1 (2%)	42 (84%)
Cattle	0 (0%)	7 (14%)	0 (0%)	10 (20%)
Sheep	1 (2%)	8 (16%)	0 (0%)	10 (20%)
Total	6 (3%)	65 (32.5%)	1 (0.5%)	74 (37%)

\*SXT: Trimothoprim/Sulfamethoxazole, AMC: Amoxicillin/Klavulanic Acid, PRL: Piperacillin, AMP: Ampicillin

**Table 6.** Antimicrobial resistance of *E. coli* isolates from human and animals**Tablo 6.** İnsan ve hayvanlardan izole edilen *E. coli* antimikrobiyel direnci

Specimen	No. (%) of Isolates Resistant to Antimicrobial Agents						
	Quinolones				Chloramphenicol	Tetracyclines	
	NOR* (10 µg)	CIP* (5 µg)	NA* (30 µg)	LEV* (5 µg)	C* (30 µg)	TE* (30 µg)	DOX* (30 µg)
Human	7 (14%)	4 (8%)	12 (24%)	3 (6%)	7 (14%)	25 (50%)	7 (14%)
Chicken	23 (46%)	8 (16%)	40 (80%)	11 (22%)	23 (46%)	24 (48%)	20 (40%)
Cattle	2 (4%)	0 (0%)	6 (12%)	0 (0%)	6 (12%)	21 (42%)	10 (20%)
Sheep	1 (2%)	1 (2%)	2 (4%)	1 (2%)	8 (16%)	10 (20%)	13 (26%)
Total	33 (16.5%)	13 (6.5%)	60 (30%)	15 (7.5%)	44 (22%)	80 (40%)	50 (25%)

\*NOR: Norfloxacin, CIP: Ciprofloxacin, NA: Nalidixic Acid, LEV: Levofloxacin, C: Chloramphenicol, TE: Tetracycline, DOX: Doxycycline

originated strains, SXT ( $P < 0.001$ ) resistance in cattle originated strains, K ( $P < 0.078$ ) and GM ( $P < 0.001$ ) resistance in sheep originated strains and SXT ( $P = 0.003$ ) resistance in human originated strains (Table 6).

## DISCUSSION

Nowadays, usage of antibiotics in farm animals is quite prevalent and widespread, and has been a typical practice of farmers all around the world [19]. The majority of drugs are fed to animals as feed additives to promote their growth in factory farms and in veterinary hospitals and as pharmaceuticals for animals. There are great amounts of data suggesting that consistently administering antibiotics to farm animals has caused an increase in the antibiotic-resistant bacteria especially in human [20]. Bacteria in the human microbiome can learn how to resist against more drugs because human are exposed to slight amounts of antibiotics than animals [21,22].

To check the role of drugs in bacterial resistance, we chose a common bacterium exposed to different antibiotics in different specimens and tested the resistance both phenotypically and genetically. Our results demonstrated that chicken isolates were highly resistant to antimicrobial agents commonly used as feed additives or therapeutics. In other words, chicken which were prescribed soltrim (trimetoprim-sulphamethoxazol), fozbac, tetracycline, doxycycline, chloramphenicol, enrofloxacin, gentamicin, furazolidone and colistin showed high resistance almost to all antibiotics specifically CTX (56%), CF (94%), TOB (72%), NOR (46%), NA (80%), AMC (68%), AMP (84%), C (46%), TE (48%) and DOX (40%) and higher integrons (Class I and II) prevalence rather than other sources (Table 6).

Regarding the results listed in Table 3, considerable resistance to cefotaxime, kanamycin, gentamicin, sulphamethoxazole/trimethoprim, norfloxacin, ciprofloxacin, nalidixic acid, levofloxacin, amoxicillin-clavulanate, ampicillin, chloramphenicol among *E. coli* isolates of chicken origin can be observed in comparison to other origins. Besides the above-mentioned results, the chicken was the only

source, which took SXT and Gentamicin. It can be observed that 10% and 18% of the chicken-derived isolates were resistant to these agents, which urges the hypothesis of antibiotic mediated resistance. Just 5 chicken and 1 sheep isolates showed resistance to folate pathway inhibitors, suggesting that SXT is an efficient antimicrobial agent. Like other categories, resistance to quinolones was more prevalent in chicken isolates, while ciprofloxacin had the most efficiency and nalidixic acid proved to be a weak antibiotic.

Our results confirm Kang *et al.* [23] survey in which commensal *E. coli* strains isolated from enrofloxacin and norfloxacin medicated chicken were compared to *E. coli* from swine which were not fed by the mentioned above agents, whereby they found the *E. coli* of chicken origin to be much more resistant than the swine ones.

In addition, Ojeniyi [24] tested 3444 *E. coli* isolates from battery hens (received antibiotic) and 2284 isolates from free-range (antibiotic free) chickens and found all isolates from battery hens as MDR, while no free-range chicken was MDR.

Van den Bogaard *et al.* [25] followed a similar study; they compared the AMR of laying hens (which were seldom given any antibiotics), broilers and turkey (with high dose drug). Finally they found the prevalence and degree of antibiotic resistance for nearly all antibiotics tested was significantly higher in the turkey and broiler population, as compared to that of the laying hens.

Sáenz *et al.* [26] investigated AMR of 474 *E. coli* isolates recovered from animal faeces (broilers, pigs, pets, bulls, and horses), human stool (patients and healthy volunteers) and food products of animal origin. They found different patterns of AMR, for example Sáenz found high frequency of nalidixic acid, ciprofloxacin and gentamicin resistance in *E. coli* isolates from broilers (88, 38 and 40%, respectively), and from foods (53, 13 and 17%, respectively). High levels of resistance to trimethoprim-sulphamethoxazole and tetracycline have been found in *E. coli* isolates from broilers, pigs, and foods. Regarding the results, they believed

there should be a significant association between drug consumption and attributed AMR, which is in accordance with our results.

Besides the phenotype results, we gained expectable genetic results, since 50% and 26% of chicken origin isolates harboured Class I and II integrase genes, respectively, while 7 isolates carried both of them. Six of 7 isolates carrying both *int I* and *II* were resistant to 6 categories indicating the role of integrons in resistance.

As a result of universal intensive chicken antibiotic feeding, the rates of AMR and integrons prevalence are to some extent similar. For example Cavicchio *et al.*<sup>[27]</sup> isolated 299 *E. coli* from avian source and found 49.8% of the isolates carried Class I and 10.4% carried Class II.

Ponce-Rivas *et al.*<sup>[28]</sup> studied *E. coli* isolates from chicken litter and found Class I integron genes in 52.63% of the isolates, which is compatible to our results. The results of these mentioned studies are in accordance to our investigation. Vasilakopoulou *et al.*<sup>[29]</sup> evaluated the prevalence of Class I integron in *E. coli* of poultry and human origin and they found the integron carriage rate for poultry isolates was 49.2%, for hospital isolates was 26.2% and for healthy people was 11.1%. Vasilakopoulou survey confirms our results (poultry integron rate) and shows a difference between AMR of hospitalized and healthy people which can be attributed to drug consumption by hospitalized patients.

Kang *et al.*<sup>[23]</sup> conducted a project to compare the integron prevalence in *E. coli* isolates with different antibiotic patterns from poultry, clinical isolates from human, healthy human and commensal isolates from swine while he found the prevalence as follow: 44%, 33%, 23% and 13%, respectively, which means the lowest and the highest incidence of integrons belongs to healthy people and poultry, respectively.

Of course the prevalence of Class I integron investigated by Oosterik *et al.*<sup>[30]</sup> is half of our report since they found *int I* gene just in 21.6% of *E. coli* isolated from poultry faeces; however, in their report there is no demographic record of antibiotic prescription to explain such difference.

Over several decades, to varying degrees, bacteria causing common infections have developed resistance to each new antibiotic, and AMR has evolved to become a worldwide health threat. This study showed that the use of antibiotics is strongly associated with the prevalence of AMR in commensal *E. coli*. Class I integrons were found to be widely disseminated among *E. coli* isolates from chicken with intensive antibiotic prescription and play pivotal role in resistance mediating. Regarding the demographic data, we can come to the conclusion that high doses of antibiotic make selection pressure and remove the susceptible microorganisms permitting the resistant to

stay and reside, such phenomenon can be accelerated by lateral transfer of integrons.

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## COMPETING INTERESTS

The authors of this article declare that there is no competing interest.

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