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Identification of integrons and phylogenetic groups of drug-resistant *Escherichia coli* from broiler carcasses in China

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ABSTRACT

The dissemination of drug-resistant *Escherichia coli* in poultry products is becoming a public concern, as it endangers food security and human health. It is very common for *E. coli* to exhibit drug resistance in the poultry industry in China due to the excessive use of antibiotics. However, few studies have examined the drug resistance endowed by integrons and integron-associated gene cassettes in different phylogenetic groups of *E. coli* isolated from broiler carcasses. In this study, 373 antibiotic-resistant *E. coli* strains were isolated from the surfaces or insides of broiler carcasses from a slaughterhouse in Shandong Province, China. According to phylogenetic assays of *chuA*, *yjaA*, and an anonymous DNA fragment, TSPE4-C2, these isolates belong to four phylogenetic groups (A, B1, B2, and D) and seven subgroups (A_0, A_1, B1, $B2_1$, $B2_2$, D_1, and D_2). Of the tested isolates, 95.71% (n = 357) are multi-drug resistant, among which group B1 was predominant, accounting for 33.51% (n = 125) of the tested isolates. A high percentage of the *E. coli* isolates were resistant to amoxicillin–clavulanic acid (99.20%, n = 370), doxycycline (92.23%, n = 344), sulfamethoxazole–trimethoprim (90.88%, n = 339), ciprofloxacin, (64.61%, n = 241), sulbactam–cefoperazone (51.21%, n = 191), and amikacin (33.78%, n = 126). Furthermore, among the 373 isolates, class 1 and 2 integrons were identified in 292 (78.28%) and 49 (13.14%) of the isolates, respectively, while no class 3 integrons were detected. The most prevalent gene cassette arrays were *dfrA17–aadA5* and *dfrA12–orfF–aadA2* in the variable region of class 1 integrons, while only one gene cassette array (*dfrA1–sat2–aadA1*) was detected in the variable region of class 2 integrons. Class 1 integrons were distributed in various physiological subtypes, whereas no predominant phylogenetic groups could be identified. The presence of class 2 integrons in the $B2_1$ subtype was significantly higher than in the other subtypes, and it coexisted with the class 1 integron. This study suggests that broiler products are potential sources of multi-drug resistant *E. coli*, and that resistance genes could be spread by lateral gene transfer.

1. Introduction

Foodborne or opportunistic pathogens may cause illnesses in humans who consume contaminated, raw poultry products. The excessive use of antibiotics in the poultry industry increases the prevalence of multidrug-resistant bacteria (Van den Bogaard et al., 2001). During broiler slaughter and processing, antibiotic-resistant bacteria spread quickly due to cross-contamination. This creates a potential public health risk in that reservoirs of antibiotic resistance (AR) genes in food sources may be acquired by human pathogens as a result of horizontal gene transfer (Martínez, 2008). Processed raw broiler carcasses are often contaminated with *Escherichia coli* (Bensink and Botham, 1983; Turtura et al., 1990). *E. coli* is a typical Gram-negative bacterium that causes colibacillosis, a common disease in chicken farms. Multidrug-resistant *E. coli* with broad-spectrum drug resistance causes great difficulties in the prevention and treatment of the disease in the poultry industry. Based on differences in genotypic and phenotypic properties, *E. coli* is divided into four phylogenetic groups, namely groups A, B1, B2, and D. The strains are categorized based on the presence of three genomic DNA sequences that can be amplified and identified via PCR: the *chuA* and *yjaA* genes, as well as the DNA fragment TSPE4-C2. *E. coli* with the *chuA*, *yjaA*, and TSPE4-C2 genotypes (−/−/−, respectively) (=A0) and −/+/− (=A1) belong to group A, −/−/+ belongs to group B1, +/+/− (=$B2_1$) and +/+/+ (=$B2_2$) belong to group B2, whereas +/−/− (=D1) and +/−/+ (=D2) belong to group D (Figueira et al., 2011). Environmental isolates or commensalisms are most often found in groups A and B1, while pathogens, such as

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verotoxin-producing *E. coli* O157:H7, are predominantly found in groups B2 and D (Clermont et al., 2000; Gordon et al., 2008; Picard et al., 1999; Pupo et al., 1997).

Integrons are genetic units that possess the capacity to capture antibiotic resistance genes, known as gene cassettes, by site-specific recombination, and they are associated with multiple drug-resistant phenotypes (Cambray et al., 2010; Hall and Collis, 1995; Stokes and Hall, 1989). Integrons are widely distributed in Gram-negative and Gram-positive bacteria, although they are predominant in the former. They play an important role in the transmission of drug resistance genes among bacteria. Thus far, five classes of integrons have been described (Mazel, 2006). Class 1 and 2 integrons are usually associated with drug-resistant *E. coli* (Han et al., 2012). A typical class 1 integron has a 5′ conserved segment and a 3′ conserved segment. The 5′ conserved segment consists of the integrase gene (*intI1*), the promoters P_{int} and P_c that are responsible for the transcription of the integrase and gene cassettes, and a recombination site (*attI*). The 3′ conserved segment usually contains the *qacE* gene, the *sul1* gene, and a variable region. Class 2 integrons are often found in transposon Tn7. The sequence of its integrase gene (*intI2*) is similar to that of *intI1*, but it is terminated prematurely and does not encode a fully functional integrase. The gene cassettes in integrons consist of a single gene and a recombination site. Several cassettes may be inserted into the same integron to form a tandem gene cassette array. Most gene cassettes in integrons contain antibiotic resistance genes (Fluit and Schmitz, 1999; Hall and Collis, 1995; Partridge et al., 2009).

Several studies have investigated *E. coli* phylogenetic groups in human and animal wastewater or other surface waters, as well as in the internal organs and feces of animals (Ahmed et al., 2005; Fogarty et al., 2003; Higgins et al., 2007; Johnson et al., 2005). Our previous study showed that every *E. coli* phylogenetic group isolated from hospital wastewater and river water in Jinan, China exhibited antibiotic resistance. Groups B2 and D, which are usually pathogenic bacteria, were more prevalent in hospital wastewater. Integrons were closely correlated with the prevalence of antibiotic resistance in *E. coli* isolated from aqueous environments (Han et al., 2012). Based on the research of Han et al. (2012), 25 different gene cassettes were found in *E. coli* isolated from hospital wastewater or river water, 22 of which harbored antibiotic resistance genes in integrons. In this study, 373 drug-resistant *E. coli* were isolated from a commercial broiler slaughter plant over a four-month period in Shandong Province, China. Their antibiotic resistance, phylogenetic subgroup affiliation (A_0, A_1, B1, $B2_1$, $B2_2$, D_1, and D_2), and the presence of integrons and integron-associated gene cassettes were identified. This is the first study of the correlation between phylogenetic subgroups and the presence of integrons in drug-resistant *E. coli* from broiler products.

2. Methods

2.1. Sampling and bacterial isolation

Samples were taken from a commercial broiler slaughter plant in Shandong Province, China, in June 2013. Sterile, moistened swabs were used to wipe 2 cm^2 areas from the surfaces and insides of 89 eviscerated broiler chicken carcasses by the method of Chaslus-Dancla and Lafont (1985). Swabs were agitated in 10 ml of sterile saline (0.9% NaCl), and the suspensions were serially diluted. A 100 μl aliquot of each dilution was spread onto eosin methylene blue (EMB) plates (AOBOX, Beijing, China) that were supplemented with one of six antimicrobial agents (Sigma-Aldrich, St. Louis, MO, USA), and the applied concentrations were based on Clinical Laboratory Standards Institute (CLSI) guidelines M07-A8 (2009), as follows: amoxicillin–clavulanic acid (AMC, 32/16 μg/ml), ciprofloxacin (CIP, 4 μg/ml), amikacin (AMK, 32 μg/ml), doxycycline (DC, 16 μg/ml), sulbactam–cefoperazone (SBT/CPZ, 64 μg/ml), or sulfamethoxazole–trimethoprim (SXT, 4/76 μg/ml). Based on colony color and morphology, individual colonies were selected and transferred to 3 ml of LB broth (AOBOX) containing the same antibiotic and incubated at 37 °C with shaking for 18 h. One milliliter of culture was stored in 25% glycerol at −80 °C, and genomic DNA was extracted from the remainder of the culture using bacterial genome extraction kits (BioTeke Corp., Beijing, China); DNA concentrations were quantified prior to use in further studies.

2.2. Antibiotic susceptibility assay

Determination of the minimum inhibitory concentration (MIC) using the E-test (AB Biodisk, Solna, Sweden) was performed for six antimicrobial agents: AMC, CIP, AMK, DC, SBT/CPZ, and SXT using Mueller-Hinton agar (MHA) plates (AOBOX) according to CLSI guidelines M07-A8 (2009). The reference strain *E. coli* ATCC 25922 was used as a quality control.

2.3. Phylogenetic subgroup assay

Drug-resistant *E. coli* phylogenetic subgroups were determined by the method of Clermont et al. (2000). Primers targeting *chuA*, *yjaA*, and TSPE4.C2 (Table 1) were used to identify the presence of these fragments in purified DNA extracted from each isolate using PCR to determine the phylogenetic groups to which each isolate belongs (Clermont et al., 2000). The detection of the three amplicons, confirmed by sequencing, served as the basis for the phylogenetic subgroup determination. The PCR amplicon pattern defining each group (*chuA*/*yjaA*/TSPE4.C2) was as follows: −/−/−, A_0; −/+/−, A_1; −/−/+, B1; +/+/−, $B2_1$; +/+/+, $B2_2$; +/−/−, D_1; and +/−/+, D_2 (Figueira et al., 2011; Han et al., 2012). To determine whether the A_0 subgroup was *E. coli*, the presence of *lacZ* genes was determined, as recommended by Higgins et al. (2007).

2.4. Occurrence of integrons and associated gene cassettes in antibiotic-resistant E. coli strains

The primer pairs IntI1F/R, IntI2F/R and IntI3F/R were used to amplify *intI1*, *intI2*, and *intI3* (Table 1). Additionally, primers hep58/59 and hep74/51 were used to amplify gene cassettes of class 1 and class 2 integrons, respectively (Han et al., 2012; White et al., 2000). The PCR program was set as follows: an initial denaturation at 94 °C for 5 min; followed by 30 cycles of 94 °C for 30 s, 40 °C for 30 s, and 72 °C for 1 min, followed by a final extension step at 72 °C for 10 min.

Furthermore, plasmids pJR88 containing *intI1* (Kagan and Davies, 1980; Wohlleben et al., 1989; Xu et al., 2007), pIP1100 containing *intI2* (Biskri and Mazel, 2003; Xu et al., 2007), and pAV3.5 containing *intI3* (Xu et al., 2007) were used as positive controls for the PCRs.

2.5. Sequencing of gene cassette arrays

The gene cassette arrays of class 1 and 2 integrons were analyzed by the PCR-based restriction fragment length polymorphism (PCR-RFLP) method, as recommended by Guo et al. (2011). PCR amplification products of the variable regions of integrons were digested with EcoRII (TaKaRa Biotechnology Co., Ltd., Dalian, China). PCR amplicons with novel RFLP patterns were cloned into the pMD19-T vector (TA clone kit; TaKaRa Biotechnology Co., Ltd.) and transformed into *E. coli* DH5α for sequencing.

2.6. Statistics analysis

Statistical analysis was performed using the chi-square test (SPSS 17.0 for Windows; SPSS Inc., Chicago, IL, USA). p-Values ≤ 0.05 were considered to be significant.

Table 1
Oligonucleotide primer pairs used in this study.

Primer	Nucleotide sequence (5′-3′)	Target gene	PCR product (bp)	References
chuA.1	GACGAACCAACGGTCAGGAT	*chuA*	279	Figueira et al. (2011)
chuA.2	TGCCGCCAGTACCAAAGACA			
YjaA.1	TGAAGTGTCAGGAGACGCTG	*yjaA*	211	Figueira et al. (2011)
YjaA.2	ATGGAGAATGCGTTCCTCAAC			
TspE4.2C-F	GAGTAATGTCGGGGCATTCA	*TspE4.2C*	364	Figueira et al. (2011)
TspE4.2C-R	CGCGCCAACAAAGTATTACG			
IntI1F	GTTCGGTCAAGGTTCTGG	*intI1*	890	Xu et al. (2007)
IntI1R	CGTAGAGACGTCGGAATG			
hep58	TCATGGCTTGTTATGACTGT	Variable region of class 1 integrons	variable	White et al. (2000)
hep59	GTAGGGCTTATTATGCACGC			
IntI2-F	CAAGCATCTCTAGGCGTA	*intI2*	1056	Xu et al. (2007)
IntI2-R	AGTAGCATCAGTCCATCC			
Hep74	CGGGATCCCGGACGGCATGCACGATTTGTA	Variable region of class 2 integrons	variable	Han et al. (2012)
Hep51	GTAGCCATCGCAAGTACGAG			
IntI3-F	CATCAAGCTGCTCGATCA	*intI3*	878	Xu et al. (2007)
IntI3-R	ACAACTCTTGCACCGTTC			

3. Results

3.1. Phylogenetic group analysis of antibiotic-resistant E. coli strains

In total, 373 drug-resistant *E. coli* strains were isolated from samples taken from broiler carcasses. Among them, 81 isolates were from EMB plates containing SXT, followed in descending order by AMC (n = 76), DC (n = 71), CIP (n = 56), AMK (n = 48), and SBT/CPZ (n = 41). Antimicrobial susceptibility tests identified that 0.54% of the isolates were resistant to one antibiotic, 3.75% were resistant to two antibiotics, and 95.71% were resistant to at least three antibiotics.

Phylogenetic assays revealed that isolated *E. coli* strains belong to four different *E. coli* groups (A, B1, B2, and D) and seven subgroups (A_0, A_1, B1, $B2_1$, $B2_2$, D_1, and D_2). A high percentage (33.51%, n = 125) of isolates belong to subgroup B1, while 19.03% (n = 71), 15.82% (n = 59), 9.38% (n = 35), and 12.87% (n = 48) of the isolates belong to subgroups A_0, A_1, D_1, and D_2, respectively (Fig. 1). Furthermore, 6.17% (n = 23) and 3.22% (n = 12) of the isolates belong to subgroups $B2_2$ and $B2_1$, respectively.

3.2. Antibiotic resistance and occurrence of integrons in antibiotic-resistant E. coli strains

Antibiotic susceptibility tests were performed on the 373 *E. coli* isolates identified in this work, and they showed that a high percentage of the *E. coli* isolates were resistant to AMC (99.20%), DC (92.23%), SXT (90.88%), CIP (64.61%), SBT/CPZ (51.21%), and AMK (33.78%) (Table 2).

We analyzed the presence of class 1, 2, and 3 integrons in antibiotic-resistant isolates. Among the 373 isolates, class 1 and 2 integrons were found in 292 (78.28%) and 49 (13.14%) isolates, respectively, while no class 3 integrons were detected. In addition, a high percentage (67.35%) of class 2 integrons coexisted with class 1 integrons.

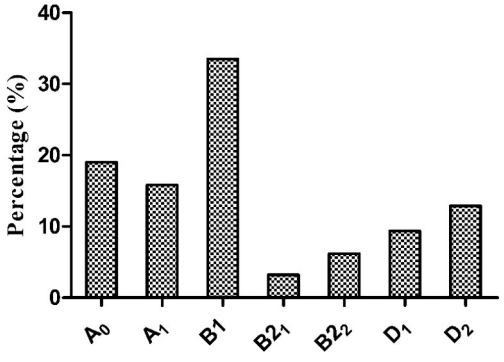

Fig. 1. Phylogenetic distribution of the isolated antibiotic-resistant *E. coli* strains.

3.3. Gene cassette analysis of class 1 and 2 integrons

Among the 292 class 1 integron-positive isolates, class 1 variable regions were successfully amplified from 199 isolates. RFLP-PCR and DNA sequencing results of the inserted gene cassettes identified 11 types of class 1 integrons containing 13 different gene cassettes (Fig. 2, Table 3). Genes identified in these antimicrobial resistance gene cassettes include: the dihydrofolate reductase genes *dfrA1*, *dfrA12*, *dfrA17*, and *dfrA27* that confer resistance to trimethoprim; the aminoglycoside adenyltransferase genes *aadA1*, *aadA2*, *aadA5*, and *aadA16* or the aminoglycoside acetyltransferase gene *aacA4* that confer resistance to streptomycin and spectinomycin; the chloramphenicol acetyltransferase gene *catB3* or the chloramphenicol efflux protein precursor gene *cmlA1* that confer resistance to chloramphenicol; the rifampin ADP-ribosylating transferase gene *arr3* that confers resistance to rifampicin; and a hypothetical protein gene (*orfF*). Dihydrofolate reductase (*dfr*) genes conferring trimethoprim resistance were the most common gene cassettes (Table 3).

The most common gene cassette arrays of the class 1 integrons were *dfrA17–aadA5* (n = 83) and *dfrA12–orfF–aadA2* (n = 74), which are consistent with the results of previous studies (Han et al., 2012; Guo et al., 2011). In 16 class 1 integron-positive isolates, no gene cassettes were identified. Two different gene cassette arrays were found to coexist in nine isolates: *dfrA17–aadA5*/*dfrA12–orfF–aadA2* (n = 7) and *dfrA17–aadA5*/*aadA2* (n = 2).

In contrast, among the 373 isolates, only 49 isolates contained class 2 integrons, in which the variable regions of the class 2 integron were successfully amplified in 46 (93.88%) of the isolates. Only one gene cassette array (*dfrA1–sat2–aadA1*), which contains the three conserved resistance gene cassettes *dfrA1*, *sat2*, and *aadA1* that confer resistance to trimethoprim, streptothricin, and streptomycin/spectinomycin, respectively, was detected in these class 2 integron-positive isolates (Fig. 2).

As shown in Table 2, all seven subgroups had a high level of resistance to AMC, DC, and SXT, while subgroups B1, $B2_2$, and D_2 displayed higher resistance to AMK. In addition, subgroup $B2_1$ showed the highest level of antimicrobial resistance to CIP. Meanwhile, subgroups A_1, $B2_2$, and D_2 had lower levels of resistance to SBT/CPZ.

The prevalence of class 1 integron-positive isolates was similar in different subgroups (Fig. 3). However, class 2 integrons were more frequently detected in subgroup $B2_1$ than in the other subgroups (42%, $p < 0.01$) (Fig. 3). Additionally, all class 2 integrons in subgroup $B2_1$ coexisted with class 1 integrons. No class 2 integrons were detected in subgroup $B2_2$.

Table 2
Resistance of isolated *E. coli* strains to tested antimicrobial agents.

Antimicrobial agent	No. of the *E. coli* isolates in each phylogenetic group that are resistant to antimicrobial agents							
	A_0 (n = 71)	A_1 (n = 59)	B1 (n = 125)	$B2_1$ (n = 12)	$B2_2$ (n = 23)	D_1 (n = 35)	D_2 (n = 48)	∑n (%)
AMC	70	58	125	12	22	35	48	370 (99.20%)
CIP	41	31	87	10	17	25	30	241 (64.61%)
AMK	20	9	56	3	10	5	23	126 (33.78%)
DC	64	48	119	11	22	33	47	344 (92.23%)
SBT/CPZ	42	22	72	8	7	19	21	191 (51.21%)
SXT	61	53	118	11	20	32	44	339 (90.88%)

Amoxicillin and potassium clavulanate, AMC; ciprofloxacin, CIP; amikacin, AMK; doxycycline, DC; sulbactam–cefoperazone, SBT/CPZ; sulfamethoxazole–trimethoprim, SXT.

4. Discussion

Antimicrobial agents are widely used in feeding additives and therapeutics, which has resulted in an increase in multidrug-resistant bacteria. In this study, we isolated 373 drug-resistant *E. coli* strains from a commercial broiler poultry plant, and examined the presence of integrons and integron-associated gene cassette arrays in these isolates.

The results obtained from this work revealed that the broiler poultry plant contained many drug-resistant *E. coli* strains, of which groups A (A_0, A_1) and B1 were more predominant than group B2 and D strains (Fig. 1); these results are consistent with previous reports of antibiotic-resistant *E. coli* from hospital wastewater and rivers, surface waters, and environmental and animal samples (Figueira et al., 2011; Han et al., 2012; Higgins et al., 2007). However, Gordon et al. (2008) reported that the frequency of subgroup A_0 was much lower (<9%) than those of group B2 and D strains isolated from soil, water, and sediment samples in Australia. It seems likely that subgroup B2 and group D strains, which are more likely to be pathogenic, are less likely to survive in the environment.

The data from the antibiotic susceptibility tests showed that 95.71% of the isolates had resistance to at least three antimicrobial agents. The high prevalence of multidrug-resistant bacteria in food is considered to be due to the overuse and misuse of antibiotics in commercial broiler poultry farms (Aminov and Mackie, 2007; Biyela et al., 2004; Martínez, 2008).

AMC is an antibiotic comprising a β-lactam antibiotic (amoxicillin trihydrate) and a β-lactamase inhibitor (clavulanic acid). This combination results in significant efficacy against amoxicillin-resistant bacteria that produce β-lactamase. A surprisingly large proportion (99.2%) of the *E. coli* strains isolated in this work are resistant to AMC, possibly because of the overproduction of β-lactamase or loss of sensitivity to the β-lactamase-inhibiting moiety clavulanic acid (Walter-Toews et al., 2011). This disturbing phenomenon indicates a decrease in the efficacy of AMC in the repression of β-lactam-resistant *E. coli*, which could lead to their potential spread in the poultry industry, possibly as a result of the abuse of β-lactam type antibiotics.

The isolates in different phylogenetic subgroups of *E. coli* showed different antibiotic resistance patterns. Compared with the other subgroups, subgroups B1, $B2_2$, and D_2 displayed higher resistance to AMK. As described previously (Han et al., 2012), groups B2 and D may have a higher resistance to all antimicrobial agents. Therefore, considering that most pathogenic *E. coli* strains are in these two groups, these results are of great concern.

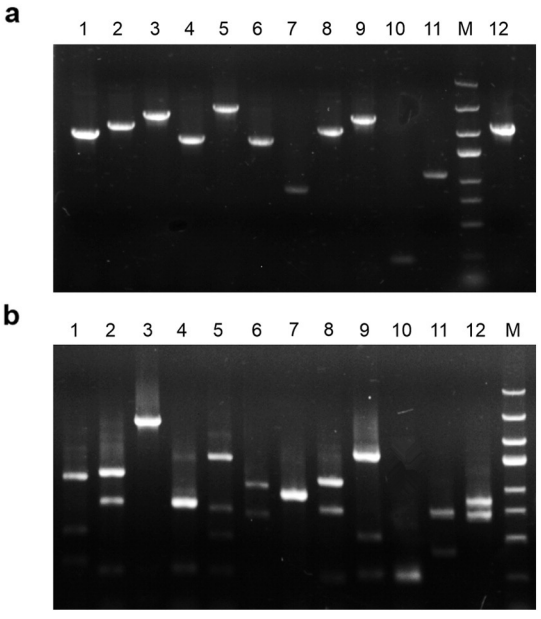

Fig. 2. RFLP patterns of gene cassette arrays in identified integrons. Panel a, products of PCRs amplifying the variable regions of integrons. Panel b, EcoRII-digested RFLP patterns. Lanes 1–11 are type 1 integrons with the following gene cassettes: *dfrA17–aadA5*; *dfrA12–orfF–aadA2*; *catB3–arr3*; *dfrA1–aadA1*; *aacA4–cmlA1*; *dfrA16–aadA2*; *dfrA17*; *arr3–dfrA27–aadA16*; *aacA4*; *aadA2*; and empty, respectively. Lane 12 is a type 2 integron with a *dfrA1–sat2–aadA1* gene cassette. M: molecular size marker. From top to bottom, each band indicates DNA sizes (in bp) of 5000, 3000, 2000, 1500, 1000, 750, 500, 250, and 100.

Table 3
Distribution of gene cassette arrays in isolates from different *E. coli* subgroups.

Subgroups		Gene cassette arrays in Class 1 integrons	Number	Gene cassette arrays in Class 2 integrons	Number
A	A_0	*dfrA17–aadA5*	14	*dfrA1–sat2–aadA1*	8
		dfrA12–orfF–aadA2	13		
		dfrA1–aadA1	2		
		aacA4–cmlA1	1		
	A_1	*dfrA17–aadA5*	12	*dfrA1–sat2–aadA1*	5
		dfrA12–orfF–aadA2	11		
		dfrA1–aadA1	6		
		Empty	4		
B1		*dfrA17–aadA5*	32	*dfrA1–sat2–aadA1*	24
		dfrA12–orfF–aadA2	26		
		dfrA1–aadA1	6		
		catB3–arr3	3		
		aacA4–cmlA1	1		
		aadA2	1		
		Empty	5		
B2	$B2_1$	*dfrA17–aadA5*	5	*dfrA1–sat2–aadA1*	5
		dfrA12–orfF–aadA2	2		
		Empty	3		
	$B2_2$	*dfrA17–aadA5*	6		
		dfrA12–orfF–aadA2	7		
		dfrA1–aadA1	2		
		catB3–arr3	4		
D	D_1	*dfrA17–aadA5*	6	*dfrA1–sat2–aadA1*	2
		dfrA12–orfF–aadA2	9		
		dfrA1–aadA1	3		
		arr3–dfrA27–aadA16	1		
	D_2	*dfrA17–aadA5*	9	*dfrA1–sat2–aadA1*	2
		dfrA12–orfF–aadA2	5		
		dfrA1–aadA1	1		
		catB3–arr3	4		
		dfrA16–aadA2	1		
		dfrA17	1		

Empty, no gene cassette was detected in the integrons.

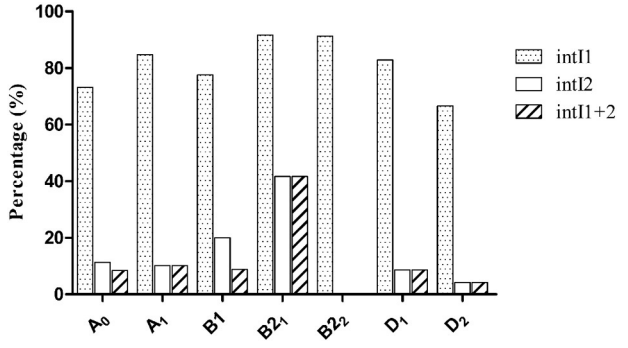

Fig. 3. The prevalence of class 1 or class 2 integrons in different *E. coli* phylogenetic groups. intI1, class 1 integron-positive isolates; intI2, class 2 integron-positive isolates; intI1 + 2, isolates positive for both class 1 and 2 integrons.

In this study, among the 373 tested isolates, class 1 and 2 integrons were identified in 292 (78.28%) and 49 (13.14%) isolates, respectively. Our results showed that the presence of integrons was more widespread than that previously reported in southwest Nigeria, where only 14% of *E. coli* isolated from poultry farms were positive for class 1 integrons and 17% were positive for class 2 integrons (Adelowo et al., 2014). This is an indication that class 1 integrons were more prevalent in antibiotic-resistant *E. coli* at the broiler poultry plant in Shandong Province, China.

Eleven types of class 1 integrons with different gene cassettes arrays were detected in the tested isolates. All of the identified genes located in the variable region of class 1 integrons were antibiotic resistance genes. In contrast, only one type of class 2 integron containing a gene cassette array (*dfrA1–sat2–aadA1*), the most classical type (Ahmed and Shimamoto, 2015), was detected in these isolates. The inability to detect type 3 integrons is not a surprise, as class 3 integrons are rarely detected, and only a few have been studied in detail (Xu et al., 2007).

The most common gene cassette arrays of the class 1 integrons detected in this study were *dfrA17–aadA5* (n = 83) and *dfrA12–orfF–aadA2* (n = 74). In addition, dihydrofolate reductase genes (*dfr*) and aminoglycoside adenyltransferase genes (*aad*), conferring resistance to trimethoprim and streptomycin, respectively, occurred frequently in the variable regions of class 1 integrons. This could be the result of the overuse or misuse of trimethoprim and streptomycin in feeding additives and therapeutics in commercial broiler poultry farms (Marshall and Levy, 2011).

Because of the presence of an internal stop cordon (TAA) in the class 2 integron integrase (*intI2*) gene, the truncated class 2 integrase cannot efficiently catalyze gene cassette integration and excision. As a result, when compared with class 1 integrons, the diversity of gene cassettes located in the variable region of class 2 integrons was much lower (Ramírez et al., 2010; Wei et al., 2014). In the 49 class 2 integron-positive isolates in this study, 33 coexisted with class 1 integrons. This high rate of coexistence is consistent with the results of our previous study (Xia et al., 2013), which showed that 63.16% of class 2 integrons coexisted with class 1 integrons. As suggested by Hansson et al. (2002), the *intI2* gene of class 2 integrons, which bears a premature stop codon, might be complemented *in trans* by class 1 integrases.

5. Conclusion

This study links the phylogenetic diversity of integrons and integron-associated gene cassettes to the prevalence of antibiotic resistance in *E. coli* isolates from a broiler poultry plant. These results indicate that broiler poultry may serve as a reservoir of antimicrobial drug resistance genes. The fact that *E. coli* strains from broiler carcasses contain an abundance of integrons and antibiotic resistance genes is a cause for concern. Our results strongly point to the critical need for the control of antimicrobial drug usage in the livestock production.

Conflict of interest

The authors declare that they have no conflicts of interest.

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