Integron-Associated Antibiotic Resistance Patterns in *Escherichia coli* Strains Isolated from Human and Animal Sources in Two Provinces of Iran

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Escherichia coli is recognized as a major food-borne pathogen of humans and animals world-wide. The strains of *E. coli* have become increasingly resistant to antibiotics, partly as a result of genes carried on integrons.

The aim of the study was to investigate the association between the existence of integrons and antibiotic resistance in *E. coli* strains isolated from human and animal sources in the Alborz and Isfahan provinces of Iran.

Materials and Methods. Twenty samples were collected from cattle and sheep at Isfahan province and poultry and humans at Alborz province. *E. coli* was isolated from these samples using standard biochemical and bacteriological techniques. Antibiotic resistance and sensitivity were determined using the Kirby–Bauer disk diffusion method. A duplex polymerase chain reaction was used to amplify the *Int1* and *Int2* genes of class 1 and 2 integrons.

Results. A total of 33 from 80 isolates (41.25%) contained integron-associated genes. Among these, 25 isolates (31.25%) harbored class 1 integrons; while 8 isolates (10.0%) contained class 2 integrons. Resistance to more than 6 antimicrobial agents was observed among the integron-positive strains.

Conclusion. Our findings showed that integrons were widely spread among *E. coli* isolated in the Alborz province. Thus, regular surveillance and monitoring of antimicrobial drug resistance in humans and animals in Iran should be performed and should include molecular screening for integrons.

Key words: integrons; antibiotic resistance; *Escherichia coli.*

Introduction

Escherichia coli is a cosmopolitan bacterium existing either as a commensal or pathogenic to humans and various animal species [1–5]. This organism has been reported to be responsible for significant veterinary, public health and socio-economic concerns in various countries worldwide [6–8].

The indiscriminate, unauthorized and unsupervised administration of antimicrobials in human and animal therapy has been suggested to be a predisposition for the dissemination of resistance genes among bacteria. This abuse of antibiotics during cattle breeding constitutes a serious threat to human and animal health because of the high risk of the selection of antibiotic resistance genes in the microorganisms [8–9].

Antimicrobial resistance determinants are carried mainly by genetic components such as plasmids, transposons, and integrons. Several authors have associated the integrons and conjugative plasmids with the spread of the resistance determinants from [9–12].

Integrons are genetic structures containing a site-specific recombination system that enables bacteria to acquire and express cassettes of genes that carry antibiotic resistance [13–15]. Integrons are transposition defective; however, they can be mobilized in association with functional transposons and/or conjugative plasmids [13]. They also contain a site-specific recombination system able to capture and express genes as gene cassettes [16, 17]. The essential components of class 1 integrons are a) the 5'conserved segment (5'-CS) that includes the integrase gene, *intI*, which encodes the site-

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specific recombinase, b) the adjacent site, *attI*, that is recognized by the integrase and acts as a receptor for gene cassettes, and c) a common promoter region(s), $P_{ant}(P_1)$ and/or $P_{ant}(P_2)$, from which the integrated gene cassettes are expressed [18, 19]. The 3'conserved segment (3'-CS) located downstream of the integrated gene cassettes, usually contains a combination of the three genes, *qacE1* (responsible for antiseptic resistance), *sulI* (implicated in the resistance to sulfonamides), and the open reading frame (*orf5*) whose function is currently uncertain [20].

Several studies have demonstrated the mechanism of clonal spread of resistant strains, transfer of resistance genes between bacteria living in humans and animals and the exchange of phylogenetic and genotypic characteristics [21]. The exponential increase and spread of antimicrobial-resistant bacteria are of a great concern because of the difficulty in treating the bacteria-borne infections. Such complications often result from rapid expansion of antibiotic-resistant genes carried by plasmids, transposons, and integrons [17, 22–24]. Several studies have reported on widespread prevalence of integrons in clinical bacteria isolates [25–28]. Therefore, the increased drug resistance of clinical isolates may be explained by the selective pressure of antibiotic and the widespread presence of integrons.

To our knowledge, there is little information on the presence of integrons in *E. coli* isolates and the association between integrons and antimicrobial resistance. Therefore, the present study was performed to investigate the association between the existence of integrons and resistance to antimicrobial agents in *E. coli* strains isolated from human and animal sources in the Alborz and Isfahan provinces, Iran.

Materials and Methods

Study location and description. This study was conducted in two locations: the city of Karaj, located in the Alborz province, and the city of Zavareh, located in the Isfahan province of Iran. The Alborz province has 2.413 million populations, and the Isfahan province has 1.6 million populations.

The present study was approved by the Ethics Committee of the Karaj Branch, Islamic Azad University.

A total of 80 samples were collected from cattle (n=20), sheep (n=20), poultry (n=20), and humans (n=20). Faeces were collected from apparently healthy people who willingly submitted their samples to the Amini Medical Laboratory located at the Alborz province. Large intestinal swabs were collected from randomly selected chickens bred in a privately owned breeding farm in Karaj city. Faeces were collected per rectum from randomly selected cattle and sheep managed in a privately owned livestock facility located in Zavareh city. No animal was hurt during sample collection in this study.

Isolation of E. coli from samples. The faecal samples were inoculated into lauryl sulphate tryptose (LST) broth (Merck, Germany) followed by inoculation into EC medium (Merck) at 44.5°C, and then passaged on eosin methylene blue (EMB) agar (Merck). Colonies with metal shine were presumed to be *E. coli* isolates; these underwent IMViC test for confirmation [29].

Antibiotic susceptibility testing. Phenotypic antibiotic susceptibility was tested for by the Kirby–Bauer disk diffusion method. Padtan-Teb disks (Tehran, Iran) were placed on Mueller–Hinton agar plates according to the guidelines of the Clinical and Laboratory Standards Institute. The 11 antibiotic discs included ampicillin (AM) 10 µg, piperacillin (PIP) 100 µg, cefazolin (CZ) 30 µg, streptomycin (SM) 10 µg, kanamycin (K) 30 µg, gentamicin (GM) 10 µg, neomycin (N) 30 µg, tobramycin (TOB) 10 µg, amikacin (AN) 30 µg, nalidixic acid (NA) 30 µg, and sulfamethoxazole/trimethoprim (SXT) 23.75/1.25 µg. For antimicrobial susceptibility testing, the inoculum of *E. coli* was homogenized with a sterile swab in sterile saline solution (0.85% NaCl) to adjust turbidity to match the 0.5 McFarland standards. These were then placed evenly on Mueller–Hinton agar plates.

The plates were inverted and then incubated at 35°C for 18 h; the diameters of the growth inhibition zones were measured and compared with the standard chart and with the *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 as positive controls. Isolates with intermediate resistance were defined as susceptible; the isolates were considered multi-drug-resistant if they were resistant to at least three classes of antibiotics [30–32].

Amplification of integrons by PCR

DNA extraction. Two colonies of each bacteria isolates were placed into a tube containing 100 µl of double-distilled water. Tubes were heated at 100°C for 10 min, and then the cells were pelleted by centrifugation. The supernatant containing DNA was taken out and stored at –20ºC [29].

Duplex PCR reaction for E. coli isolates. All *E. coli* isolates were tested by multiplex PCR using previously described conditions and protocols [33]. Two sets of primers were used to amplify the 287 and 789 bp fragments of the *int1* and *int2* genes respectively (Table 1). Duplex PCR reaction was performed in a

Table 1

Two sets of primers used in the multiplex PCR reaction

Target gene	Forward primer (5'→3')	Reverse primer (5'→3')	Size (bp)
Int1	TCTCGGGTAACATCAAGG	GTTCTTCTACGGCAAGGT	287
Int2	CACGGATATGCGACAAAAAGGT	GTAGCAAACGAGTGACGAAATG	789

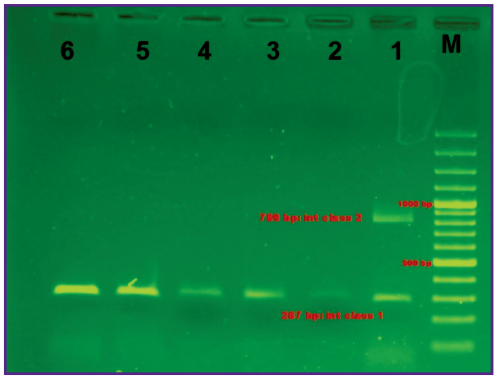
25 µl reaction mixture, containing PCR buffer (10 mM Tris-HCl, 50 mM KCl, and 1.5 mM $MgCl_2$, pH 8.7), dNTP (200 µM), a primer (0.4 µM), Taq DNA polymerase (1 U), and template DNA (2 µl). The PCR reaction was performed in a DNA thermocycler (Model CP2-003; Corbett, Australia) as follows: initial denaturation — at 94°C for 4 min, 30 cycles of denaturation — at 94°C for 5 s, annealing — at 59°C for 10 s, elongation — at 72ºC for 30 s and the final extension step — at 72°C for 5 min, followed by cooling at 4°C. PCR products were electrophoresed on 1.5% agarose gel containing ethidium bromide at 80 volts for 1 h.

Results

In this study, 33 of 80 *E. coli* isolates (41.25%) contained integron-associated genes. Among these, 25 isolates (31.25%) harbored class 1 integrons. Class 1 integrons were identified in the strains recovered from sheep (n=4), chickens (n=12), cows (n=1), and humans (n=8). Eight isolates (10.0%) contained class 2 integrons which were identified in the strains recovered from sheep (n=1), chickens (n=6), and humans (n=1) (see Figure).

Most of the *E. coli* strains isolated from sheep, chickens, cows, and humans were resistant to piperacillin, tobramycin, amikacin, and gentamicin while a lower percentage showed resistance to cefazolin and nalidixic acid (Table 2).

In sheep, class 1 integrons were detected in four sulfamethoxazole/trimethoprim-resistant *E. coli* isolates and class 2 integrons were detected in one sulfamethoxazole/trimethoprim-resistant *E. coli* isolate (Table 3). On the other hand, class 1 and class 2 integrons were detected in twelve and six multiple drug resistance *E. coli* isolates from chickens, respectively (Table 4). In cows, only class 1 integron was detected in an *E. coli* isolate with streptomycin and sulfamethoxazole/trimethoprim resistance (Table 5). In one of eight *E. coli* isolates from humans, class 1 integrons and class 2 integrons were detected. Multiple



Detection of *int1 and int2* genes in *E. coli* strains. Lane M, 100 bp marker scale; lanes 1–6, positive strains

Table 2

Patterns of *E. coli* resistance to antibiotics

Antibiotics (concentration on disks)	Sources			
	Sheep (n=20)	Chickens (n=20)	Cows (n=20)	Humans (n=20)
CZ (30 µg)	6	9	0	8
AM (10 µg)	5	12	7	7
PIP (100 µg)	0	1	0	0
SM (10 µg)	9	13	17	19
TOB (10 µg)	0	5	0	0
SXT (23.75/1.25 µg)	4	11	2	9
AN (30 µg)	0	0	0	0
NA (30 µg)	0	15	2	6
GM (10 µg)	0	5	0	0
K (30 µg)	2	3	1	2
N (30 µg)	3	5	1	2

Here: CZ: cefazolin, AM: ampicillin, PIP: piperacillin, SM: streptomycin, TOB: tobramycin, SXT: sulfamethoxazole/trimethoprim, AN: amikacin, NA: nalidixic acid, GM: gentamicin, K: kanamycin N: neomycin.

Table 3

Types of drug resistance in *E. coli* from sheep

Source (sheep)	*Int1*	*Int2*	CZ	AM	PIP	SM	TOB	SXT	AN	NA	GM	K	N	Antimicrobial resistance pattern
1	—	—	I	I	S	R	I	S	I	S	I	I	I	SM
2	+	—	R	I	S	I	I	R	I	S	I	I	I	CZ, SXT
3	+	—	R	R	I	I	S	R	I	S	S	I	I	CZ, AM, SXT
4	—	—	I	S	S	R	I	S	I	S	I	I	I	SM
5	—	—	I	I	S	I	I	S	I	S	I	I	I	—
6	—	—	I	I	S	R	I	S	I	S	I	R	R	SM, K, N
7	—	—	I	I	S	R	S	S	S	S	S	S	I	SM
8	—	—	I	I	S	I	I	S	I	S	I	I	I	—
9	+	+	I	I	S	I	I	R	I	S	I	I	I	SXT

The end of Table 3

Source (sheep)	*Int1*	*Int2*		CZ	AM	PIP	SM	TOB	SXT	AN	NA	GM	K	N	Antimicrobial resistance pattern
10	—	—		R	I	S	R	I	S	I	S	S	S	S	CZ, SM
11	—	—		I	S	S	I	I	S	I	S	I	I	I	—
12	—	—		R	R	S	R	S	S	I	I	I	I	I	CZ, AM, SM
13	—	—		I	I	S	I	I	S	I	S	S	I	R	N
14	—	—		R	R	I	R	I	I	S	S	S	S	S	CZ, AM, SM
15	—	—		I	I	S	I	I	S	I	S	S	S	I	—
16	—	—		I	S	S	R	I	S	I	S	S	I	I	SM
17	—	—		I	S	I	I	I	S	I	S	S	I	I	—
18	—	—		I	R	S	R	S	S	I	S	I	I	I	AM, SM
19	—	—		R	R	I	I	I	S	I	S	I	R	R	CZ, AM, K, N
20	+	—		I	I	S	I	I	R	I	I	I	I	I	SXT
Total	4	1	R	6	5	0	9	0	4	0	0	0	2	3	
			S	0	4	16	0	4	15	2	18	8	4	2	
			I	14	11	4	11	16	1	18	2	12	14	15	

N o t e: R: resistance; I: intermediate resistance; S: susceptibility. Drug abbreviations see Table 2.

Table 4

Comparison of antibiotic resistance patterns in *E. coli* from chickens

Source (chickens)	*Int1*	*Int2*		CZ	AM	PIP	SM	TOB	SXT	AN	NA	GM	K	N	Antimicrobial resistance pattern
1	+	+		R	R	I	R	R	R	I	R	R	I	I	CZ, AM, SM, TOB, SXT, NA, GM
2	+	—		R	R	S	R	I	R	I	R	R	I	I	CZ, AM, SM, SXT, NA, GM
3	+	+		I	S	S	I	I	R	S	R	I	I	I	SXT, NA
4	+	—		I	I	S	I	I	S	I	S	I	I	R	N
5	—	+		I	R	S	R	I	I	S	S	S	I	S	AM, SM
6	+	—		I	R	S	R	R	R	S	R	R	I	R	AM, SM , TOB, SXT, NA, GM, N
7	+	—		R	R	S	R	I	R	S	R	S	I	I	CZ, AM, SM , SXT, NA
8	—	+		I	I	S	I	S	R	S	R	S	I	I	SXT, NA
9	—	—		R	R	R	R	S	S	S	R	I	S	I	CZ, AM, PIP, SM, NA
10	—	—		I	I	S	I	I	S	I	R	S	I	I	NA
11	+	—		R	R	I	I	R	R	I	R	R	R	I	CZ, AM, TOB, SXT, NA, GM, K
12	+	—		I	R	S	R	R	R	S	R	R	I	I	AM, SM, TOB, SXT, NA, GM
13	+	+		R	R	S	R	R	R	S	R	I	I	R	CZ, AM, SM, SXT, NA, K, N
14	—	—		I	S	S	R	I	S	I	R	I	I	I	SM, NA
15	+	—		I	S	I	R	I	S	I	R	I	I	I	SM, NA
16	+	—		R	R	S	R	S	R	S	R	S	R	R	CZ, AM, SM, SXT, NA, K, N
17	—	—		I	I	S	R	S	S	I	R	I	I	I	SM, NA
18	+	+		R	R	S	R	S	R	S	S	S	I	S	CZ, AM, SM, SXT
19	—	—		R	R	S	I	I	S	I	S	I	R	R	CZ, AM, K, N
20	—	—		I	I	S	I	S	S	I	R	I	S	S	NA
Total	12	6	R	9	12	1	13	5	11	0	16	5	3	5	
			S	0	3	16	0	6	8	10	4	6	2	3	
			I	11	5	3	7	9	1	10	0	9	15	12	

N o t e: R: resistance; I: intermediate resistance; S: susceptibility. Drug abbreviations see Table 2.

Table 5

Comparison of antibiotic resistance patterns in *E. coli* from cows

Source (cows)	*Int1*	*Int2*		CZ	AM	PIP	SM	TOB	SXT	AN	NA	GM	K	N	Antimicrobial resistance pattern
1	—	—		S	I	S	R	S	R	S	R	I	I	S	SM, SXT, NA
2	—	—		S	R	S	R	S	S	S	S	S	S	S	AM, SM
3	—	—		S	I	S	R	S	S	I	S	S	I	I	SM
4	—	—		S	I	S	R	S	S	S	S	S	S	I	SM
5	—	—		S	R	S	R	S	S	S	S	S	S	R	AM, SM, N
6	—	—		S	I	S	R	S	S	S	S	S	I	R	SM, K
7	—	—		S	R	S	R	S	S	S	S	S	S	I	AM, SM
8	—	—		S	I	S	R	S	S	S	S	S	S	I	SM
9	—	—		S	I	S	R	S	S	S	S	S	S	I	SM
10	—	—		I	I	S	R	S	S	S	S	S	S	S	SM
11	—	—		I	R	S	R	S	S	S	S	S	S	S	AM, SM
12	—	—		S	R	S	I	S	S	S	S	S	S	I	AM
13	—	—		S	R	S	R	S	S	S	S	S	S	S	AM, SM
14	—	—		S	I	S	I	S	S	S	S	S	S	I	—
15	—	—		S	S	S	I	S	S	S	R	S	S	S	NA
16	+	—		I	I	S	R	S	R	S	S	S	S	S	SM, SXT
17	—	—		I	S	S	R	S	S	S	S	S	S	S	SM
18	—	—		S	R	S	R	S	S	S	S	S	S	I	AM, SM
19	—	—		S	I	S	R	S	S	S	S	S	S	I	SM
20	—	—		I	I	S	R	S	S	S	S	S	S	R	SM
Total	1	0	R	0	7	0	17	0	2	0	2	0	0	3	
			S	15	2	20	0	20	18	19	18	19	17	8	
			I	5	11	0	3	0	0	1	0	1	3	9	

N o t e: R: resistance; I: intermediate resistance; S: susceptibility. Drug abbreviations see Table 2.

Table 6

Comparison of antibiotic resistance patterns in *E. coli* from humans

Source (humans)	*Int1*	*Int2*	CZ	AM	PIP	SM	TOB	SXT	AN	NA	GM	K	N	Antimicrobial resistance pattern
1	—	—	I	I	S	R	S	S	S	S	S	S	S	SM
2	+	—	R	R	S	R	S	R	S	S	S	S	S	CZ, AM, SM, SXT
3	—	—	I	I	S	R	S	S	S	S	S	S	S	SM
4	—	—	R	I	S	R	S	S	I	S	S	S	I	CZ, SM
5	+	—	I	S	S	R	I	R	S	R	I	I	I	SM, SXT, NA
6	—	—	I	I	S	R	I	S	S	S	S	S	I	SM
7	—	—	I	I	S	R	S	S	S	S	S	S	S	SM
8	—	+	R	R	S	I	I	R	S	S	S	S	S	CZ, AM, SXT
9	+	—	R	R	S	R	S	R	S	R	S	S	I	CZ, AM, SM, SXT, NA
10	—	—	I	S	S	R	S	S	S	S	S	S	S	SM
11	+	—	R	R	S	R	S	S	S	S	S	S	S	CZ, AM, SM
12	+	—	I	S	S	R	S	R	S	R	I	R	S	SM, SXT, NA, K
13	+	—	I	R	S	R	S	R	S	R	S	S	R	AM, SM, SXT, NA, N
14	—	—	I	I	S	R	I	R	S	S	S	S	I	SM, SXT
15	—	—	R	I	S	R	S	S	S	S	S	S	S	CZ, SM

The end of Table 6

Source (humans)	*Int1*	*Int2*		CZ	AM	PIP	SM	TOB	SXT	AN	NA	GM	K	N	Antimicrobial resistance pattern
16	+	—		R	R	S	R	S	R	S	R	S	S	S	CZ, AM, SM, SXT, NA
17	+	—		I	I	S	R	S	R	S	R	S	R	R	SM, SXT, NA, K, N
18	—	—		R	R	S	R	S	S	S	S	S	S	S	CZ, AM, S
19	—	—		I	S	S	R	S	S	S	S	S	S	S	SM
20	—	—		I	I	S	R	S	S	S	S	S	S	I	SM
Total	8	1	R	8	7	0	19	0	9	0	6	0	2	2	
			S	0	4	20	0	16	11	19	14	18	17	12	
			I	12	9	0	1	4	0	1	0	2	1	6	

N o t e: R: resistance; I: intermediate resistance; S: susceptibility. Drug abbreviations see Table 2.

T a b l e 7

Comparison of antibiotic resistance patterns between isolates with and without integrons (%)

Antibiotics (concentration on disks)	Total of resistant isolates (n=80) (%/abs. number)	Integron-negative (n=52)			Integron-positive (n=28)			p	
		R	I	S	R	I	S	*Int1*	*Int2*
CZ (30 μg)	28.75/23	17.4	51.9	30.7	50	50	0	SS	NS
AM (10 μg)	38.75/31	28.2	54	17.8	60.7	25	14.3	NS	NS
PIP (100 μg)	1.25/1	1.9	5.7	92.4	0	14.2	85.8	NS	NS
SM (10 μg)	72.5/58	75	25	0	67.8	32.2	0	NS	SS
TOB (10 μg)	6.25/5	0	32.6	67.4	17.8	39.3	42.9	NS	NS
SXT (23.75/1.25 μg)	32.5/26	5.7	3	91.3	82.3	3.5	14.2	SS	SS
AN (30 μg)	0/0	0	40.3	59.7	0	32.2	67.9	NS	NS
NA (30 μg)	30/24	13.4	1.9	84.7	60.7	3.5	35.8	SS	NS
GM (10 μg)	6.25/5	0	30.8	69.2	18.8	29.5	51.7	SS	NS
K (30 μg)	10/8	7.6	28.9	63.5	14.2	60.8	25	SS	SS
N (30 μg)	13.75/11	9.6	53.8	36.6	22.9	54.5	22.6	NS	NS

N o t e: R: resistance; I: intermediate resistance; S: susceptibility; NS: not statistically significant; SS: statistically significant. Drug abbreviations see Table 2.

drug resistance was also detected in majority *E. coli* isolates from humans (Table 6).

Resistance to more than six antimicrobial agents was observed among integron-positive strains (Table 7). Our findings showed that integrons were common among *E. coli* isolated in the Alborz province. Class 1 integrons prevailed over class 2 integrons.

Discussion

Resistance to antibiotics in enterobacteriaceae can be caused by mutation or action of mobile DNA elements such as plasmids, transposons, and integrons [34]. Integrons have the ability to capture antibiotic resistance genes by site-specific recombination. Based on the type of integrase gene, five integron classes have been described to date [35, 36].

There are few reports on the occurrence and activity of integrons in microorganisms. In the present study, 41.25% of the *E. coli* strains isolated from sheep, chickens, cows, and humans harbored one or two integrin-associated genes. This number (41.25%) was within the range of prevalence (22 to 59%) reported in clinical *E. coli* isolates by others [27, 37]. In some of these isolates, only one integron class was detected while others had multiple integrons. This observation suggests that integrons commonly exist in the genome of enterobacteriaceae and may be responsible for the rapid development of antibiotic resistance.

The results of our study also indicated that 31.25%

of *E. coli* isolates carried class 1 integrons; this number was higher than that reported by Tennstedt et al. [38], who detected the presence of class 1 integrons in 12.4% of resistance plasmids obtained from urban waste water. This figure (12.4%) was however lower than those reported from Norway [39], Western and Central Europe [27], Netherlands [40], France [26], Korea [41], and China [42].

Antibiotic resistance patterns found in our study showed that streptomycin-resistant bacteria could be isolated from animals, probably as a result of streptomycin and spectinomycin use in animal husbandry [43–47]. Furthermore, coliform bacteria isolated from humans became colonized with streptomycin-resistant bacteria via the food chain in a contaminated environment [48–50]. It is also possible that integrons are transferred from animal *E. coli* to human *E. coli* while transiently passing through the human intestine.

Antibiotic resistance patterns observed in the animal and human *E. coli* isolates in the present study are in line with a study from Ireland that multi-drug resistance is associated with class 1 integrons in *E. coli* serotypes isolated from soil samples and cattle faeces [51]. Several studies have also reported the presence of integrons in uropathogenic *E. coli* and have established a strong association between the presence of integrons and antimicrobial resistance in multi-drug- and single-drug-resistant *E. coli* strains [52, 53].

In the present study, class 1 and class 2 integrons were detected in twelve and six multiple drug resistance *E. coli* isolates from chicken samples, respectively. These results are similar to a recent report that found antimicrobial resistance in uropathogenic *E. coli* from Europe and Canada [52].

Conclusion

Integrons were widely disseminated among *E. coli* isolates from the Alborz province. Increased surveillance and the development of adequate prevention strategies are warranted to elucidate the diversity of factors occurring in these environments.

Authors' contributions. RR carried out the molecular genetic studies and participated in their design. HM carried out the biochemical study. NH participated in designing the study and performed the statistical analysis. RK performed the sampling and carried out the molecular genetic studies. FK conceived and coordinated the study and helped in drafting the manuscript. All authors contributed equally to this study. All authors have read and approved the final manuscript.

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References

1. Rahimi E., Khamesipour F., Yazdi F., Momtaz H. Isolation and characterization of enterohaemorragic Escherichia coli O157:H7 and EHEC O157:NM from raw bovine, camel, water buffalo, caprine and ovine milk in Iran. *Kafkas Univ Vet Fak Derg* 2012; 18(4): 559–564, https://doi.org/10.9775/kvfd.2011.5738.

2. Raissy M., Khamesipour F., Rahimi E., Khodadoostan A. Occurrence of Vibrio spp., Aeromonas hydrophila, Escherichia coli and Campylobacter spp. in crayfish (Astacus leptodactylus) from Iran. *IJFS* 2014; 13(4): 944–954.

3. Hemmatinezhad B., Khamesipour F., Mohammadi M., Safarpoor Dehkordi F., Mashak Z. Microbiological investigation of O-serogroups, virulence factors and antimicrobial resistance properties of Shiga toxin-producing Escherichia coli isolated from ostrich, turkey and quail meats. *Journal of Food Safety* 2015; 35(4): 491–500, https://doi.org/10.1111/jfs.12199.

4. Ranjbar R., Hosseini S., Zahraei-Salehi T., Kheiri R., Khamesipour F. Investigation on prevalence of Escherichia coli strains carrying virulence genes ipaH, estA, eaeA and bfpA isolated from different water sources. *Asian Pac J Trop Dis* 2016; 6(4): 278–283, https://doi.org/10.1016/s2222-1808(15)61031-3.

5. Tajbakhsh E., Ahmadi P., Abedpour-Dehkordi E., Arbab-Soleimani N., Khamesipour F. Biofilm formation, antimicrobial susceptibility, serogroups and virulence genes of uropathogenic E. coli isolated from clinical samples in Iran. *Antimicrob Resist Infect Control* 2016; 5(1): 11, https://doi.org/10.1186/s13756-016-0109-4.

6. Ranjbar R., Pezeshknejad P., Khamesipour F., Amini K., Kheiri R. Genomic fingerprints of Escherichia coli strains isolated from surface water in Alborz province, Iran. *BMC Research Notes* 2017; 10(1): 295, https://doi.org/10.1186/s13104-017-2575-z.

7. Ranjbar R., Haghi-Ashtiani M.T., Jafari N.J., Abedini M. The prevalence and antimicrobial susceptibility of bacterial uropathogens isolated from pediatric patients. *Iranian Journal of Public Health* 2009; 38(2): 134–138.

8. Afkhami Ardakani M., Ranjbar R. Molecular typing of uropathogenic E. coli strains by the ERIC-PCR method. *Electron Physician* 2016; 8(4): 2291–2295, https://doi.org/10.19082/2291.

9. Nagachinta S., Chen J. Integron-mediated antibiotic resistance in Shiga toxin–producing Escherichia coli. *J Food Prot* 2009; 72(1): 21–27, https://doi.org/10.4315/0362-028x-72.1.21.

10. Ranjbar R., Giammanco G.M., Farshad S., Owlia P., Aleo A., Mammina C. Serotypes, antibiotic resistance, and class 1 integrons in Salmonella isolates from pediatric cases of enteritis in Tehran, Iran. *Foodborne Pathog Dis* 2011; 8(4): 547–553, https://doi.org/10.1089/fpd.2010.0736.

11. Farshad S., Ranjbar R., Japoni A., Hosseini M., Anvarinejad M., Mohammadzadegan R. Microbial susceptibility, virulence factors, and plasmid profiles of uropathogenic Escherichia coli strains isolated from children in Jahrom, Iran. *Arch Iran Med* 2012; 15(5): 312–316.

12. Talebiyan R., Kheradmand M., Khamesipour F., Rabiee-Faradonbeh M. Multiple antimicrobial resistance of Escherichia coli isolated from chickens in Iran. *Vet Med Int* 2014; 2014: 491418, https://doi.org/10.1155/2014/491418.

13. Cambray G., Guerout A.-M., Mazel D. Integrons. *Annu Rev Genet* 2010; 44(1): 141–166, https://doi.org/10.1146/annurev-genet-102209-163504.

14. Tajbakhsh E., Khamesipour F., Ranjbar R., Ugwu I.C. Prevalence of class 1 and 2 integrons in multi-drug resistant Escherichia coli isolated from aquaculture water in Chaharmahal Va Bakhtiari province, Iran. *Ann Clin Microbiol Antimicrob* 2015; 14(1): 37, https://doi.org/10.1186/s12941-015-0096-y.

15. Kheiri R., Ranjbar R., Khamesipour F., Akhtari L. Role of antibiotic in drug resistance and integrons prevalence in Escherichia coli isolated from human and animal specimens. *Kafkas Univ Vet Fak Derg* 2016; 22(6): 953–959, https://doi.org/10.9775/kvfd.2016.15684.

16. Bennett PM. Integrons and gene cassettes: a genetic construction kit for bacteria. *J Antimicrob Chemother* 1999; 43(1): 1–4, https://doi.org/10.1093/jac/43.1.1.

17. Hall R.M., Collis C.M. Mobile gene cassettes and integrons: capture and spread of genes by site-specific recombination. *Mol Microbiol* 2006; 15(4): 593–600, https://doi.org/10.1111/j.1365-2958.1995.tb02368.x.

18. Collis C.M., Hall R.M. Expression of antibiotic resistance genes in the integrated cassettes of integrons. *Antimicrob Agents Chemother* 1995; 39(1): 155–162, https://doi.org/10.1128/aac.39.1.155.

19. Recchia G.D., Hall R.M. Gene cassettes: a new class of mobile element. *Microbiology* 1995; 141(12): 3015–3027, https://doi.org/10.1099/13500872-141-12-3015.

20. Paulsen I.T., Littlejohn T.G., Rådström P., Sundström L., Sköld O., Swedberg G., Skurray R.A. The 3' conserved segment of integrons contains a gene associated with multidrug resistance to antiseptics and disinfectants. *Antimicrob Agents Chemother* 1993; 37(4): 761–768.

21. Van den Bogaard A. Epidemiology of resistance to antibiotics. Links between animals and humans. *Int J Antimicrob Agents* 2000; 14(4): 327–335, https://doi.org/10.1016/s0924-8579(00)00145-x.

22. Normark B.H., Normark S. Evolution and spread of antibiotic resistance. *J Intern Med* 2002; 252(2): 91–106.

23. Momtaz H., Karimian A., Madani M., Safarpoor Dehkordi F., Ranjbar R., Sarshar M., Souod N. Uropathogenic Escherichia coli in Iran: serogroup distributions, virulence factors and antimicrobial resistance properties. *Ann Clin Microbiol Antimicrob* 2013; 12: 1–12.

24. Torkan S., Bahadoranian M., Khamesipour F., Anyanwu M. Detection of virulence and antimicrobial resistance genes in Escherichia coli isolates from diarrhoiec dogs in Iran. *Archivos de medicina veterinaria* 2016; 48(2): 181–190, https://doi.org/10.4067/s0301-732x2016000200008.

25. Gonzalez G., Sossa K., Bello H., Dominguez M., Mella S., Zemelman R. Presence of integrons in isolates of different biotypes of Acinetobacter baumannii from Chilean hospitals. *FEMS Microbiol Lett* 1998; 161(1): 125–128, https://doi.org/10.1111/j.1574-6968.1998.tb12937.x.

26. Hamada K., Oshima K., Tsuji H. Drug resistance genes encoded in integrons and in extra-integrons: their distribution and lateral transfer among pathogenic enterobacteriaceae including enterohemorrhagic Escherichia coli and Salmonella enterica serovars typhimurium and infantis. *Jpn J Infect Dis* 2003; 56(3): 123–126.

27. Martinez-Freijo P., Fluit A.C., Schmitz F.J., Grek V.S., Verhoef J., Jones M.E. Class I integrons in gram-negative isolates from different European hospitals and association with decreased susceptibility to multiple antibiotic compounds. *J Antimicrob Chemother* 1998; 42(6): 689–696, https://doi.org/10.1093/jac/42.6.689.

28. Martinez-Freijo P., Fluit A.C., Schmitz F.-J., Verhoef J., Jones M.E. Many class I integrons comprise distinct stable structures occurring in different species of Enterobacteriaceae isolated from widespread geographic regions in Europe. *Antimicrob Agents Chemother* 1999; 43(3): 686–689, https://doi.org/10.1128/aac.43.3.686.

29. Obeng A.S., Rickard H., Ndi O., Sexton M., Barton M. Antibiotic resistance, phylogenetic grouping and virulence potential of Escherichia coli isolated from the faeces of intensively farmed and free range poultry. *Vet Microbiol* 2012; 154(3–4): 305–315, https://doi.org/10.1016/j.vetmic.2011.07.010.

30. Bukh A.S., Schønheyder H.C., Emmersen J.M.G., Søgaard M., Bastholm S., Roslev P. Escherichia coli phylogenetic groups are associated with site of infection and level of antibiotic resistance in community-acquired bacteraemia: a 10 year population-based study in Denmark. *J Antimicrob Chemother* 2009; 64(1): 163–168, https://doi.org/10.1093/jac/dkp156.

31. Blanco J., Mora A., Mamani R., López C., Blanco M., Dahbi G., Herrera A., Blanco J.E., Alonso M.P., García-Garrote F., Chaves F., Orellana M.Á., Martínez-Martínez L., Calvo J., Prats G., Larrosa M.N., González-López J.J., López-Cerero L., Rodríguez-Baño J., Pascual A. National survey of Escherichia coli causing extraintestinal infections reveals the spread of drug-resistant clonal groups O25b:H4-B2-ST131, O15:H1-D-ST393 and CGA-D-ST69 with high virulence gene content in Spain. *J Antimicrob Chemother* 2011; 66(9): 2011–2021, https://doi.org/10.1093/jac/dkr235.

32. Bashir S., Sarwar Y., Ali A., Mohsin M., Saeed M.A., Tariq A., Haque A. Multiple drug resistance patterns in various phylogenetic groups of uropathogenic E. coli isolated from Faisalabad region of Pakistan. *Braz J Microbiol* 2011; 42(4): 1278–1283, https://doi.org/10.1590/s1517-83822011000400005.

33. Clermont O., Bonacorsi S., Bingen E. Rapid and simple determination of the Escherichia coli phylogenetic group. *Appl Environ Microbiol* 2000; 66(10): 4555–4558, https://doi.org/10.1128/aem.66.10.4555-4558.2000.

34. Zhao S., White D.G., Ge B., Ayers S., Friedman S., English L., Wagner D., Gaines S., Meng J. Identification and characterization of integron-mediated antibiotic resistance among Shiga toxin-producing Escherichia coli isolates. *Appl Environ Microbiol* 2001; 67(4): 1558–1564, https://doi.org/10.1128/aem.67.4.1558-1564.2001.

35. Collis C.M., Kim M.-J., Partridge S.R., Stokes H.W., Hall R.M. Characterization of the class 3 integron and the site-specific recombination system it determines. *J Bacteriol* 2002; 184(11): 3017–3026, https://doi.org/10.1128/jb.184.11.3017-3026.2002.

36. Sallen B., Rajoharison A., Desvarenne S., Mabilat C. Molecular epidemiology of integron-associated antibiotic resistance genes in clinical isolates of Enterobacteriaceae. *Microb Drug Resist* 1995; 1(3): 195–202, https://doi.org/10.1089/mdr.1995.1.195.

37. Fluit A.C., Schmitz F.J. Class 1 integrons, gene cassettes, mobility, and epidemiology. *Eur J Clin Microbiol Infect Dis* 1999; 18(11): 761–770, https://doi.org/10.1007/s100960050398.

38. Tennstedt T., Szczepanowski R., Braun S., Pühler A., Schlüter A. Occurrence of integron-associated resistance gene cassettes located on antibiotic resistance plasmids isolated from a wastewater treatment plant. *FEMS Microbiol Ecol* 2003; 45(3): 239–252, https://doi.org/10.1016/s0168-6496(03)00164-8.

39. Heir E., Lindstedt B.A., Leegaard T.M., Gjernes E., Kapperud G. Prevalence and characterization of integrons in blood culture Enterobacteriaceae and gastrointestinal Escherichia coli in Norway and reporting of a novel class 1 integron-located lincosamide resistance gene. *Ann Clin Microbiol Antimicrob* 2004; 3: 12.

40. Jones M.E., Peters E., Weersink A.-M., Fluit A., Verhoef J. Widespread occurrence of integrons causing multiple antibiotic resistance in bacteria. *Lancet* 1997; 349(9067): 1742–1743, https://doi.org/10.1016/s0140-6736(05)62954-6.

41. Yu H.S., Lee J.C., Kang H.Y., Ro D.W., Chung J.Y., Jeong Y.S., Tae S.H., Choi C.H., Lee E.Y., Seol S.Y., Lee Y.C., Cho D.T. Changes in gene cassettes of class 1 integrons among Escherichia coli isolates from urine specimens collected in Korea during the last two decades. *J Clin Microbiol* 2003; 41(12): 5429–5433, https://doi.org/10.1128/jcm.41.12.5429-5433.2003.

42. Su J., Shi L., Yang L., Xiao Z., Li X., Yamasaki S. Analysis of integrons in clinical isolates of Escherichia coli in China during the last six years. *FEMS Microbiol Lett* 2006; 254(1): 75–80, https://doi.org/10.1111/j.1574-6968.2005.00025.x.

43. Ridley A., Threlfall E.J. Molecular epidemiology of antibiotic resistance genes in multiresistant epidemic Salmonella typhimurium DT 104. *Microb Drug Resist* 1998; 4(2): 113–118.

44. McDonald L.C., Chen M.T., Lauderdale T.L., Ho M. The use of antibiotics critical to human medicine in food-producing animals in Taiwan. *J Microbiol Immunol Infect* 2001; 34(2): 97–102.

45. Lindstedt B.-A. Characterization of class I integrons in clinical strains of Salmonella enterica subsp. enterica serovars Typhimurium and Enteritidis from Norwegian hospitals. *J Med Microbiol* 2003; 52(2): 141–149, https://doi.org/10.1099/jmm.0.04958-0.

46. Du X., Shen Z., Wu B., Xia S., Shen J. Characterization of class 1 integrons-mediated antibiotic resistance among calf pathogenic Escherichia coli. *FEMS Microbiol Lett* 2005; 245(2): 295–298, https://doi.org/10.1016/j.femsle.2005.03.021.

47. Kang H.Y., Jeong Y.S., Oh J.Y., Tae S.H., Choi C.H., Moon D.C., Lee W.K., Lee Y.C., Seol S.Y., Cho D.T., Lee J.C. Characterization of antimicrobial resistance and class 1 integrons found in Escherichia coli isolates from humans and animals in Korea. *J Antimicrob Chemother* 2005; 55(5): 639–644, https://doi.org/10.1093/jac/dki076.

48. Wegener H.C., Aarestrup F.M., Jensen L.B., Hammerum A.M., Bager F. Use of antimicrobial growth promoters in food animals and Enterococcus faecium resistance to therapeutic antimicrobial drugs in Europe. *Emerg Infect Dis* 1999; 5(3): 329–335, https://doi.org/10.3201/eid0503.990303.

49. Sanchez S., McCrackin Stevenson M.A., Hudson C.R., Maier M., Buffington T., Dam Q., Maurer J.J. Characterization of multidrug-resistant Escherichia coli isolates associated with nosocomial infections in dogs. *J Clin Microbiol* 2002; 40(10): 3586–3595, https://doi.org/10.1128/jcm.40.10.3586-3595.2002.

50. Guerra B. Phenotypic and genotypic characterization of antimicrobial resistance in German Escherichia coli isolates from cattle, swine and poultry. *J Antimicrob Chemother* 2003; 52(3): 489–492, https://doi.org/10.1093/jac/dkg362.

51. Scott L., McGee P., Walsh C., Fanning S., Sweeney T., Blanco J., Karczmarczyk M., Earley B., Leonard N., Sheridan J.J. Detection of numerous verotoxigenic E. coli serotypes, with multiple antibiotic resistance from cattle faeces and soil. *Vet Microbiol* 2009; 134(3–4): 288–293, https://doi.org/10.1016/j.vetmic.2008.08.008.

52. Blahna M.T., Zalewski C.A., Reuer J., Kahlmeter G., Foxman B., Marrs C.F. The role of horizontal gene transfer in the spread of trimethoprim–sulfamethoxazole resistance among uropathogenic Escherichia coli in Europe and Canada. *J Antimicrob Chemother* 2006; 57(4): 666–672, https://doi.org/10.1093/jac/dkl020.

53. Rao A.N., Barlow M., Clark L.A., Boring J.R. 3rd, Tenover F.C., McGowan J.E. Jr. Class 1 integrons in resistant Escherichia coli and Klebsiella spp., US hospitals. *Emerg Infect Dis* 2006; 12(6): 1011–1014.