High incidence of multidrug resistance and class 1 and 2 integrons in Escherichia coli isolated from broiler chickens in South of Iran

Mohsen Kalantari, Hassan Sharifyazdi*, Keramat Asasi, Bahman Abdi-Hachesoo

Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

Abstract

The objective was to investigate the multidrug resistance and presence of class 1 and 2 integrons in 300 Escherichia coli isolates obtained from 20 broiler farms during three rearing periods (one-day-old chicks, thirty-day-old chickens, and one day before slaughter) in Fars, South Iran. Results showed that 81.00%, 82.00%, and 85.00% of isolates were multidrug-resistant on the first day, thirty-day-old chickens, and one day before slaughter, respectively. Multidrug-resistant E. coli isolates were further examined for the presence of class 1 and 2 integrons using PCR assay. The existence of class 1 integron-integrase gene (intI1) was confirmed in 68.40%, 72.70%, and 60.90% of multidrug-resistant isolates from stage 1, stage 2, and stage 3 of the rearing period, respectively. The frequency of class 2 integron-integrase gene (intI2) during the first to the third stage of sampling was 2.60%, 25.50%, and 30.40%. Also, sequence analysis of the cassette arrays within class 1 integron revealed the presence of the genes associated with resistance for trimethoprim (dfrA), streptomycin (aadA), erythromycin (ereA), and orfF genes. The results revealed that percentages of antimicrobial resistance in E. coli isolates were significantly higher in the middle and end stages of the rearing period. In conclusion, widespread dissemination of class 1 integrons in all three stages and rising trends of class 2 integrons existence in E. coli isolates during the rearing period of broiler chickens could exacerbate the spread of resistance factors among bacteria in the poultry industry. Future research is needed to clarify its implication for human health.

Introduction

Over the past decades, the rising trend of antimicrobials as growth promoters and prophylactic and therapeutic agents in the poultry industry has caused a challenging issue of increasing bacterial antimicrobial resistance.1 The broad application of antimicrobials in chickens has increased the risk of bacterial resistance, and the administration of more than one antimicrobial during the rearing period of chickens may cause the dissemination of antimicrobial resistance, especially in gram-negative bacteria such as Escherichia coli.2 Selective pressure resulting from the use of antimicrobials in the poultry industry is found to be associated with multiple-drug resistance (MDR) in both commensal and pathogenic E. coli.3 This phenomenon is not only resulted from the natural ability of bacteria to survive and proliferate to more numbers but also related to horizontal gene transfer through plasmids.4 Multidrug resistance in enteric organisms such as E. coli has been recognized to be related to integrons.5 Integrons are bacterial genetic platforms that can increase the uptake and gene expression in their gene cassettes.6 Integrons classification is generally based on the sequence of integrase protein that gives them recombination ability. Up to date, four general classes of integrons have been recognized and distinguished, out of which most of the studies have been done on class 1, and 2.5 Class 1 integrons are widely disseminated among Gram-negative bacteria in humans and animals. This class is the most common class of Integrons in clinical isolates and so is called clinical integrons.7 More than 130 different gene cassettes have been identified in different integrons classes, which cause

*Correspondence:
Hassan Sharifyazdi, DVM, PhD
Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
E-mail: sharify@shirazu.ac.ir

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resistance to almost all antimicrobials.\textsuperscript{8} Integron gene cassettes that have been identified to date are typically encoding antimicrobial resistance factors and are known as Resistance Integrons (RIs) or Multi-drug Resistance Integrons (MRIs).\textsuperscript{9} Adjacent resistance genes on integrons have been stated to encode MDR and get involved in transferring this situation in bacteria.\textsuperscript{10} The presence of integrons has been reported in multidrug-resistant commensal and pathogenic \textit{E. coli} isolated from chicken farms worldwide.\textsuperscript{11-16} In Iran, preliminary studies showed a high prevalence of phenotypic and genotypic resistance to fluoroquinolones and tetracyclines in \textit{E. coli} isolated from broiler chickens during different stages of a rearing period.\textsuperscript{19,20} Accordingly, this study was aimed to determine the incidence of MDR for intestinal \textit{E. coli} isolated from broiler chickens during the rearing period and distribution of class 1 integron-integrase gene (\textit{intD1}) and class 2 integron-integrase gene (\textit{intD2}) in these isolates.

Materials and Methods

Sampling. Samples were taken from 20 broiler chicken flocks from different farms located in Fars province, South of Iran. Sampling was done in three stages of the rearing period: one-day-old broiler chicks on arrival at the farms, 30 days of age, and one day before slaughter (42-47 days old). This study complied with the Ethical Principles in Animal Research, approved and performed in accordance with the ethical standards of the Committee for Animal Experiments of School of Veterinary Medicine, Shiraz University (IACUC no: 4687/63). The research was conducted under the permission of a farm owner who gave informed consent for the study to be carried out. Five pooled cloacal swabs were taken from each farm in glass tubes containing tryptic soy broth (TSB) medium (Merck, Darmstadt, Germany) and instantly transferred to the laboratory.

Isolation and identification of \textit{E. coli}. Tryptic soy broth medium cultures were cultured on MacConkey agar (Merck) plates and incubated overnight at 37.00 °C. A pink colored distinct colony from each plate was used to culture on the Eosin Methylene Blue (EMB) agar (Merck) plates and incubated for 24 hr at 37.00 °C. Greenish metallic colonies on EMB agar were considered \textit{E. coli} and identified using standard biochemical tests (Gram stain, oxidase test, TSI test, indole test, citrate test, methyl red and Voges-Proskauer tests, and urea agar test).\textsuperscript{21} Confirmed isolates were deposited in TSB medium with 30.00% glycerol at -70.00 °C.

Antimicrobial susceptibility test. \textit{E. coli} isolates were tested for susceptibility to nalidixic acid (NAL, 30.00 µg), flumequine (FM, 30.00 µg), ciprofloxacin (CIP, 5.00 µg), enrofloxacin (ENR, 5.00 µg), norfloxacin (NOR, 10.00 µg), ampicillin (AM, 10.00 µg), furazolidone (FR, 100 µg), gentamicin (GM, 10.00 µg), neomycin (N, 30.00 µg), lincompectin (LP, 200/15.00 µg), tetracycline (TET, 30.00 µg), oxytetracycline (T, 30.00 µg), chloramphenicol (C, 30.00 µg), florfenicol (FF, 30.00 µg), erythromycin (E, 15.00 µg), streptomycin (S, 10.00 µg), and trimethoprim-sulfamethoxazole (SXT, 23.75/1.25 µg) on Mueller-Hinton agar (Merck) by the disc diffusion method (antibiotic discs: PadtanTeb, Tehran, Iran) as described in the National Committee for Clinical and Laboratory Standards.\textsuperscript{22,23} The \textit{E. coli} ATCC 25922 reference strain was used as the quality control.

The presence of multiple resistance. Recently standardized criteria developed by European Centre for Disease Prevention and Control (ECDC) were used to define Multidrug-resistant (MDR) \textit{E. coli} isolates (non-susceptible to ≥3 classes of drugs), extensively drug-resistant (XDR) \textit{E. coli} isolates (non-susceptible to all but 1 or 2 classes of drugs), and pandrug-resistant (PDR) \textit{E. coli} isolates (non-susceptible to all antimicrobial categories) considering the following antimicrobial categories: Aminoglycosides, penicillins, folate pathway inhibitors, phenicols, tetracyclines, and fluoroquinolones.\textsuperscript{24}

PCR amplification of integrase genes. DNA extraction was done using the boiling method.\textsuperscript{25} Integrons class 1 and 2 were investigated among 139 multidrug-resistant \textit{E. coli} isolates (38 isolates for stage one, 55 and 46 isolates for stage two and three, respectively), and, consequently, gene cassettes were explored in isolates that were positive for the \textit{intD1} gene, using PCR and direct sequencing. The primers for amplifying the \textit{intD1} gene were \textit{intM1-U} (5’-ACGAGGGAAGTTTGTCTG-3’) and \textit{intM1-D} (5’-GAAAGGTGTCATATGCAT-3’) which produced a 565 bp fragment. The primer was paired to amplify the \textit{intD2} gene (403 bp) was intM2-U (5’-GTGCAAGCATTCGATCAG-3’) and intM2-D (5’-CAACAGG AAGCTGATCAGATG-3’). Also, to identify the gene cassette element(s) in class 1, the integron, we used in-F (5’-GGCATACAGACGAGACG-3’) as the forward primer and in-B (5’-AAGCATACAGCACGAGAC-3’) as the reverse one.\textsuperscript{27} The PCR reaction (20.00 µL) was performed in 10.00 mM Tris-HCl, pH = 8.30-8.80, 50.00 mM KCl, 1.50 mM MgCl₂, 0.20 mM dNTPs, 10.00 pmol of forward and reverse primers (Gen Fanavaran Co., Tehran, Iran), 2.00 U Taq DNA polymerase, and 2.00 µL (~40.00 ng) of the DNA extract as template.

Statistical analysis. Chi-square (χ²) test was performed to evaluate the susceptibility or resistance against different antimicrobials and the frequency of integrase genes of class 1 and 2 integrons in \textit{E. coli} isolated during the three stages of sampling. This test was also used to calculate the association between antimicrobial resistance profile and integron existence. The results were statistically analyzed using the SPSS statistical software (version 16.0; SPSS Inc., Chicago, USA). Differences among means with \textit{p} < 0.05 were accepted as statistically significant.
Results

The antimicrobial susceptibility test results during the rearing period 1, 2, and 3 are presented in Table 1. The antimicrobial susceptibility test showed high-level resistance to erythromycin and streptomycin in all three stages. There were no significant differences among the stages in antimicrobial resistance ($p > 0.05$). However, resistance against other antimicrobials except ampicillin was significantly lower in day-old chicks than the middle and last days of the rearing period ($p < 0.05$). Ampicillin showed a different trend in contrast to other antimicrobials, where ampicillin resistance was significantly higher in day-old chicks ($p < 0.05$).

Overall, 82.60% of isolates were MDR and 48.00% of E. coli isolates were extensively drug resistant (XDR), and 26.00% of isolates were pandrug-resistant (PDR). The highest percentage of MDR isolates (85.00%) was seen in stage 3 (a day before slaughter). The detailed description of three stages of rearing period is as follows: Stage 1 ($n = 100$), 81.00% MDR, 54.00% XDR and 4.00% PDR; stage 2 ($n = 100$), 82.00% MDR, 45.00% XDR and 2.00% PDR; stage 3 ($n = 100$), 85.00% MDR, 45.00% XDR and 2.00% PDR.

After determining MDR isolates, all the resistant isolates to at least ten antimicrobials of the seventeen inspected antimicrobials were chosen for the detection of class 1 and class 2 integrons. According to this procedure, 139 MDR isolates were selected from three stages: 38 isolates from one-day-old chicks, 55 from 30-day-old chickens, and 46 isolates from ready to slaughter chickens. The frequencies of intI1 and intI2 genes among the three stages of sampling are shown in Table 2. In general, from all of the 139 isolates, 67.60% were positive for intI1, 20.90% for intI2, and 8.60% for both integrase 1 and 2 genes (Fig. 1A). Results showed that the presence of the intI1 gene among E. coli isolates from three stages were not significantly different ($p > 0.05$). However, intI2 gene was significantly lower in one-day-old chicks than the two later stages of the rearing period ($p < 0.05$).

Table 1. Antibiotic susceptibility test of E. coli isolates during a rearing period of broiler chickens (Stage 1: day-old chicks, Stage 2: 30-day-old, and Stage 3: A day before slaughter).

| Antimicrobials          | Stage 1 (n=100) | Stage 2 (n=100) | Stage 3 (n=100) |
|-------------------------|-----------------|-----------------|-----------------|
|                         | S (%)           | I (%)           | R (%)           | S (%)           | I (%)           | R (%)           | S (%)           | I (%)           | R (%)           |
| Nalidixic acid          | 20.00           | 3.00            | 77.00           | 8.00            | 0.00            | 92.00           | 0.00            | 0.00            | 100.00          |
| Flumequine              | 22.00           | 2.00            | 76.00           | 8.00            | 0.00            | 92.00           | 0.00            | 0.00            | 100.00          |
| Ciprofloxacin           | 58.00           | 5.00            | 37.00           | 18.00           | 5.00            | 77.00           | 12.00           | 7.00            | 81.00           |
| Enrofloxacin            | 24.00           | 30.00           | 46.00           | 15.00           | 7.00            | 78.00           | 3.00            | 7.00            | 90.00           |
| Norfloxacin             | 55.00           | 6.00            | 39.00           | 17.00           | 5.00            | 78.00           | 6.00            | 12.00           | 82.00           |
| Ampicillin              | 23.00           | 31.00           | 46.00           | 54.00           | 11.00           | 35.00           | 52.00           | 17.00           | 31.00           |
| Furazolidone            | 74.00           | 1.00            | 25.00           | 47.00           | 1.00            | 52.00           | 28.00           | 0.00            | 72.00           |
| Gentamicin              | 98.00           | 0.00            | 2.00            | 88.00           | 6.00            | 6.00            | 85.00           | 6.00            | 9.00            |
| Neomycin                | 1.00            | 43.00           | 56.00           | 2.00            | 25.00           | 73.00           | 0.00            | 16.00           | 84.00           |
| Lincomycin              | 63.00           | 20.00           | 17.00           | 49.00           | 23.00           | 28.00           | 57.00           | 10.00           | 33.00           |
| Tetracycline            | 33.00           | 0.00            | 67.00           | 7.00            | 3.00            | 90.00           | 2.00            | 4.00            | 94.00           |
| Oxytetracycline         | 33.00           | 0.00            | 67.00           | 6.00            | 0.00            | 94.00           | 4.00            | 2.00            | 94.00           |
| Chloramphenicol         | 55.00           | 0.00            | 45.00           | 38.00           | 5.00            | 57.00           | 24.00           | 0.00            | 76.00           |
| Florfenicol             | 83.00           | 1.00            | 16.00           | 51.00           | 5.00            | 44.00           | 74.00           | 5.00            | 21.00           |
| Erythromycin            | 2.00            | 0.00            | 98.00           | 0.00            | 1.00            | 99.00           | 0.00            | 1.00            | 99.00           |
| Streptomycin            | 2.00            | 53.00           | 45.00           | 0.00            | 48.00           | 52.00           | 1.00            | 64.00           | 35.00           |
| Trimethoprim-sulfa      | 64.00           | 0.00            | 36.00           | 20.00           | 3.00            | 77.00           | 17.00           | 3.00            | 80.00           |

S: susceptible; I: intermediate; R: resistant.

Fig. 1. A) PCR amplification of integrase 1 and 2 genes in E. coli isolated from broiler chickens. Lane 1: DNA marker, Lane 2: Negative control, Lane 3: Positive control of integrase 1, Lane 4: Positive control of integrase 1, Lane 5: Isolate positive for co-existence of both integrase genes, Lane 6: Isolate positive for integrase 1 gene, Lane 7: Isolate positive for integrase 2; B) PCR amplification of genes cassette in class 1 integrons. Bands with different sizes (~3200 bp, 1586 bp, 2097 bp, 1913 bp, and 1664 bp) harbor different resistance genes.
The number of integrase 1 (intI1), integrase 2 (intI2) genes, and co-existence of intI1 and intI2 genes in MDR E. coli isolated during a rearing period of broiler chickens (stage 1: one-day-old chicks, stage 2: 30-day-old and stage 3: Aday before slaughter).

| Stages | Isolates | intI1 (%) | intI2 (%) | intI1 + intI2 (%) |
|--------|----------|-----------|-----------|------------------|
| 1      | 38       | 26 (68.40) | 1 (2.60)  | 27 (65.70)       |
| 2      | 55       | 40 (72.70) | 14 (25.50) | 15 (27.30)       |
| 3      | 46       | 28 (60.90) | 14 (30.40) | 4 (8.7)          |

ab Columns with different superscripts have significant differences (p < 0.05).

Five different class 1 integron gene cassette arrays, classified as type I-V, were identified in the class 1 integron positive isolates (Fig. 1B). Four different genes were identified, including dihydrofolate reductase (dfrA), aminoglycoside adenyllyltransferase (aadA), erythromycin esterase (ereA), and a hypothetical protein (orfF), (Table 3).

Discussion

High rates of antimicrobial resistance were found in this study, even in E. coli isolated from one-day-old chicks. It has already been shown that antimicrobial-resistant E. coli isolated from broiler chickens could be inherited vertically from chicken breeders. Therefore, antimicrobial resistance rates in a non-treated broiler farm could be affected by antimicrobial treatments in their broiler breeders. Vertical transmission from broiler breeders and the acquisition of antimicrobial resistance bacterial isolates from hatcheries could introduce antimicrobial resistant bacterial clones to broiler farms. Antimicrobial resistance could increase from one-day-old chicks to ready to slaughter chickens, independent of the use of antimicrobials. However, selective pressure due to antimicrobial use during the rearing period could intensify the rate of antimicrobial resistance. Our previous work demonstrated that antimicrobial use in a rearing period was the most effective risk factor for rising fluoroquinolone resistance during a rearing period of broilers.

In the present study, MDR was high among E. coli isolates even in one-day-old chicks, and the rising trend of this resistance was not statistically different during the rearing period. The high rate of multiple drug resistance in one-day-old chicks could be a direct consequence of high resistance against some of the antimicrobials in these chicks. It has been publicized that resistance to a specific antimicrobial could dramatically shift their microbial antibiogram to a multidrug resistance profile even in the early days of chicken life. High rates of multidrug resistance persisted steadily in the E. coli isolated in our study during the middle and last day of the rearing period so that 85.00% of isolates from ready to slaughter chickens showed multidrug resistance. This too high MDR rate in E. coli isolated from pre-slaughter chickens could be challenging for human health because there is some evidence on transmission of multidrug-resistant E. coli clones, plasmids, and other transmissible elements such as integrons from poultry to humans.

The high incidence of the intI1 gene (67.60%) was found among MDR cloacal E. coli isolates in our study, while only 20.90% of isolates had the intI2 gene. Higher frequency of class 1 compared to class 2 integrons were consistent with the previous studies in commensal and pathogenic E. coli isolated from poultry, especially in chickens. In contrast, there are infrequent reports on a slightly higher incidence of intI2 gene in E. coli isolated from turkeys.

Table 3. Size and contents of gene cassettes and antibiotic resistance profile of sequenced MDR E. coli isolates.

| Sequenced samples | Cassette size (bp) | Gene cassettes | Resistance phenotype |
|-------------------|--------------------|----------------|----------------------|
| 1                 | 1586               | dfrA1, aadA1   | LP, TET, C, S, FM, SXT, E, NOR, ENR, T, NAL, CIP, FR, FF, N |
| 2                 | 1586               | dfrA1, aadA1   | LP, TET, C, S, FM, SXT, E, AM, NOR, ENR, T, NAL, CIP, FF, N |
| 3                 | 1664               | dfrA17, aadA5  | TET, C, S, FM, SXT, E, AM, NOR, ENR, T, NAL, CIP, FR, N |
| 4                 | 1913               | dfrA12, orfF, aadA2 | LP, TET, C, S, GM, FM, SXT, E, AM, NOR, ENR, T, NAL, CIP, FR, N |
| 5                 | 1586               | dfrA1, aadA1   | LP, TET, C, S, FM, SXT, E, AM, NOR, ENR, T, NAL, CIP, FR, FF, N |
| 6                 | 1664               | dfrA17, aadA5  | TET, C, S, FM, SXT, E, AM, NOR, ENR, T, NAL, CIP, FR, N |
| 7                 | 1586               | dfrA1, aadA1   | LP, TET, C, S, FM, SXT, E, AM, NOR, ENR, T, NAL, CIP, FR, FF, N |
| 8                 | ~3200              | dfrA17, ereA1, aadA2 | TET, C, S, GM, FM, SXT, E, AM, NOR, ENR, T, NAL, CIP, FR, N |
| 9                 | 1913               | dfrA12, orfF, aadA2 | LP, TET, C, S, FM, SXT, E, AM, NOR, ENR, T, NAL, CIP, FR, N |
| 10                | 1586               | dfrA1, aadA1   | LP, TET, C, S, FM, SXT, E, AM, NOR, ENR, T, NAL, CIP, FR, N |
| 11                | 2097               | dfrA5, ereA2   | LP, TET, C, S, FM, SXT, E, NOR, ENR, T, NAL, CIP, FR, FF, N |
| 12                | 1913               | dfrA12, orfF, aadA2 | LP, TET, C, S, FM, SXT, E, NOR, ENR, T, NAL, CIP, FR, N |
| 13                | 1586               | dfrA1, aadA1   | LP, TET, C, S, FM, SXT, E, NOR, ENR, T, NAL, CIP, FR, N |
| 14                | 2097               | dfrA5, ereA2   | TET, C, S, FM, SXT, E, NOR, ENR, T, NAL, CIP, FR, FF, N |
| 15                | 1586               | dfrA1, aadA1   | TET, S, FM, SXT, E, AM, NOR, ENR, T, NAL, CIP, FR, FF, N |
| 16                | ~3200              | dfrA17, ereA1, aadA2 | LP, TET, C, S, FM, SXT, E, AM, NOR, ENR, T, NAL, CIP, FR, FF, N |

NAL: Nalidixic acid, FM: Flumequine, CIP: Ciprofloxacin, ENR: Enrofloxacin, NOR: Norfloxacin, AM: Ampicillin, FR: Furazolidone, GM: Gentamicin, N: Neomycin, LP: Lincomycin, T: Tetracycline, TET: Oxytetracycline, C: Chloramphenicol, FF: Florfenicol, E: Erythromycin, S: Streptomycin, SXT: Trimethoprim-sulfamethoxazole.
Other investigations are dealing only with the presence of class 1 integrons in *E. coli* isolated from poultry of USA, China, Hungary, Korea, and Belgium, for which class 1 integrons were positive for 63.00%, 59.00%, 41.00%, 39.60%, and 21.60% of isolates, respectively. Our study results showed a high incidence of *intI1* gene among *E. coli* isolated from three stages of the rearing period, which was not significantly different between these stages. On the other hand, a significantly higher incidence of the *intI2* gene was found in the middle and last days of the rearing period. These findings could emphasize the role of the *intI2* gene in triggering, more excellent antimicrobial resistance during the second and later stages of sampling in this study.

Sequence analysis of *intI1* cassette arrays showed different types of antimicrobial resistance genes for three antimicrobials family consisting of macrolides (erythromycin), aminoglycosides (streptomycin), and folic acid synthesis inhibitors (trimethoprim), and one more gene (*orfF*), causing no known antimicrobial resistance so far. These resistance genes showed five different cassette arrangements. Soufi et al. studied 166 *E. coli* isolates recovered from poultry meat in Tunisia and showed that *aadA* (types 1, 2, and 5), *dfrA* (types 1, 12, 14, and 17), and *orfF* in five different cassette arrays were associated with class 1 integrons. Another study in China showed that 59.00% of *E. coli* isolates recovered from broiler chickens had class 1 integrons, which streptomycin and trimethoprim resistance (*dhfr1, aadA1, dhfr17, aadA2, dhfr13*) were harbored in the variable zone of them. Cavicchio et al. investigated class 1 and class 2 integrons in avian pathogenic *E. coli* from poultry in Italy and showed that *aadA1* and the combinations of *aadA1-dfrA1* and *dfrA1-aadA1* genes were the most common cassette arrays in class 1 integrons. In our study, the combination of *dfrA1* and *aadA1* was the most common gene cassettes in class 1 integrons of cloacal *E. coli* isolates. Similar results were shown by Yang et al., Kim et al., and Cocchi et al. for avian pathogenic *E. coli* isolates.

In conclusion, the present study results revealed high percentages of multi-drug resistance in commensal *E. coli* isolates from broiler chickens with the widespread dissemination of class 1 and the rising trend of class 2 integrons existence in these *E. coli* isolates during the rearing period of broiler chickens. These trends could exacerbate the spread of resistance factors among bacteria in the poultry industry and a rising global threat for public health in slaughtered chickens.

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**Conflict of interest**

The authors declare no conflicts of interest and no financial, personal, or other relationships with other people or organizations.

**References**

1. Nhung NT, Chansiripornchai N, Carrique-Mas JJ. Antimicrobial resistance in bacterial poultry pathogens: A review. Front Vet Sci 2017; 4:126. doi: 10.3389/fvets.2017.00126.
2. Simoneit C, Burow E, Tenhagen B-A, et al. Oral administration of antimicrobials increase antimicrobial resistance in *E. coli* from chicken–a systematic review. Prev Vet Med 2015; 118(1): 1-7. doi: 10.1016/j.prevetmed.2014.11.010.
3. Gyles CL. Antimicrobial resistance in selected bacteria from poultry. Anim Health Res Rev 2008; 9(2): 149-158.
4. Apata DF. Antibiotic resistance in poultry. Int J Poult Sci 2009; 8(4): 404-408.
5. Deng Y, Bao X, Ji L, et al. Resistance integrons: class 1, 2 and 3 integrons. Ann Clin Microbiol Antimicrob 2015; 14: 45. doi: 10.1186/s12941-015-0100-6.
6. Stokes HW, Hall RM. A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. Mol Microbiol 1989; 3(12): 1669-1683.
7. Hillgers MR. Class 1 integrons as invasive species. Curr Opin Microbiol 2017; 38: 10-15.
8. Mazel D. Integrons: agents of bacterial evolution. Curr Opin Microbiol 2006; 9(8): 608-620.
9. Stalder T, Barraud O, Casellas M, et al. Integron involvement in environmental spread of antibiotic resistance. Front Microbiol 2012; 3: 119. doi: 10.3389/fmicb.2012.00119.
10. Leverstein-van Hall MA, M Blok HE, T Donders AR, et al. Multidrug resistance among *Enterobacteriaceae* is strongly associated with the presence of integrons and is independent of species or isolate origin. J Infect Dis 2003; 187(2): 251-259.
11. Yang H, Chen S, White DG, et al. Characterization of multiple-antimicrobial-resistant *Escherichia coli* isolates from diseased chickens and swine in China. J Clin Microbiol 2004; 42(8): 3483-3489.
12. Nogrady N, Pászti J, Pikó H, et al. Class 1 integrons and their conjugal transfer with and without virulence-associated genes in extra-intestinal and intestinal *Escherichia coli* of poultry. Avian Pathol 2006; 35(4): 349-356.
13. Kim TE, Jeong YW, Cho SH, et al. Chronological study of antibiotic resistances and their relevant genes in Korean avian pathogenic *Escherichia coli* isolates. J Clin Microbiol 2007; 45(10): 3309-3315.
14. Dessie HK, Bae DH, Lee YJ. Characterization of integrons and their cassettes in Escherichia coli and Salmonella isolates from poultry in Korea. Poult Sci 2013; 92(11): 3036-3043.

15. Cavicchio L, Dotto G, Giacomelli M, et al. Class 1 and class 2 integrons in avian pathogenic Escherichia coli from poultry in Italy. Poult Sci 2015; 94(6): 1202-1208.

16. Li Y, Chen L, Wu X, et al. Molecular characterization of multidrug-resistant avian pathogenic Escherichia coli isolated from septicemic broilers. Poult Sci 2015; 94(4): 601-611.

17. Awad A, Arafa N, Elhadidy M. Genetic elements associated with antimicrobial resistance among avian pathogenic Escherichia coli. Ann Clin Microbiol Antimicrob 2016; 15(1): 59. doi: 10.1186/s12941-016-0174-9.

18. Moser KA, Zhang L, Spicknall I, et al. The role of mobile genetic elements in the spread of antimicrobial resistant Escherichia coli from chickens to humans in small-scale production poultry operations in rural Ecuador. Am J Epidemiol 2017; 187(3): 558-567.

19. Zibandeh S, Sharifiyazdi H, Asasi K, et al. Investigation of tetracycline resistance genes in Escherichia coli isolates from broiler chickens during a rearing period in Iran. Vet Arh 2016; 86(4): 565-572.

20. Abdi-Hachesoo B, Asasi K, Sharifiyazdi H. Farm-level evaluation of enrofloxacin resistance in Escherichia coli isolated from broiler chickens during a rearing period. Comp Clin Pathol 2017; 26: 471-476.

21. Quinn P, Carter ME, Carter GR, et al. Clinical veterinary microbiology. 2nd ed. London, UK; Wolfe Publishing 1994; 209-236.

22. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard. 4th ed. Wayne, USA: CLSI 2013.

23. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 26th ed. Wayne, USA: CLSI 2016; M100S.

24. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance Clin Microbiol Infect 2012; 18(3): 268-281.

25. Holmes DS, Quisley M. A rapid boiling method for the preparation of bacterial plasmids. Anal Biochem 1981; 114(1): 193-197.

26. Su J, Shi L, Yang L, et al. Analysis of integrons in clinical isolates of Escherichia coli in China during the last six years. FEMS Microbiol Lett 2006; 254(1): 75-80.

27. Zhang H, Shi L, Li L, et al. Identification and characterization of class 1 integron resistance gene cassettes among Salmonella strains isolated from healthy humans in China. Microbiol Immunol 2004; 48(9): 639-645.

28. Petersen A, Christensen JP, Kuhnert P, et al. Vertical transmission of a fluoroquinolone-resistant Escherichia coli within an integrated broiler operation. Vet Microbiol 2006; 116(1-3): 120-128.

29. Nilsson O, Börjeson S, Landén A, et al. Vertical transmission of Escherichia coli carrying plasmid-mediated AmpC (pAmpC) through the broiler production pyramid. J Antimicrob Chemother 2014; 69(6): 1497-1500.

30. Ozaki H, Esaki H, Takemoto K, et al. Antimicrobial resistance in faecal Escherichia coli isolated from growing chickens on commercial broiler farms. Vet Microbiol 2011; 150(1-2): 132-139.

31. Bortolai V, Biafra V, Bojesen AM. Distribution and possible transmission of ampicillin- and nalidixic acid-resistant Escherichia coli within the broiler industry. Vet Microbiol 2010; 142(3-4): 379-386.

32. Saleha AA, Myaing TT, Ganapathy KK, et al. Possible effect of antibiotic-supplemented feed and environment on the occurrence of multiple antibiotic resistant Escherichia coli in chickens. Int J Poult Sci 2009; 8(1): 28-31.

33. Dierix CM, van der Goot JA, Smith HE, et al. Presence of ESBL/AmpC-producing Escherichia coli in the broiler production pyramid: a descriptive study. PLoS ONE 2013; 8(11): e79005. doi: 10.1371/journal.pone.0079005.

34. Ginn CA, Browning GF, Benham ML, et al. Antimicrobial resistance and epidemiology of Escherichia coli in broiler breeder chickens. Avian Pathol 1996; 25(3): 591-605.

35. Witte W. Ecological impact of antibiotic use in animals on different complex microflora: environment. Int J Antimicrob Agents 2000; 14(4): 321-325.

36. Smith JL, Drum DJV, Dai Y, et al. Impact of antimicrobial usage on antimicrobial resistance in commensal Escherichia coli strains colonizing broiler chickens. Appl Environ Microbiol 2007; 73(5): 1404-1414.

37. da Costa PM, Bica A, Vaz-Pires P, et al. Effects of antimicrobial treatment on selection of resistant Escherichia coli in broiler fecal flora. Microb Drug Resist 2008; 14(4): 299-306.

38. van den Bogaard AE, London N, Driessen C, et al. Antimicrobial treatment on selection of resistant Escherichia coli in poultry, poultry farmers and poultry slaughterers. J Antimicrob Chemother 2001; 47(6): 763-771.

39. Miles TD, McLaughlin W, Brown PD. Antimicrobial resistance of Escherichia coli isolates from broiler chickens and humans. BMC Vet Res 2006; 2: 7. doi: 10.1186/1746-6148-2-7.

40. Vasilakopoulou A, Psichogiou M, Tzouvelekis L, et al. Prevalence and characterization of class 1 integrons in Escherichia coli of poultry and human origin. Foodborne Pathog Dis 2009; 6(10): 1211-1218.
41. Goldstein C, Lee MD, Sanchez S, et al. Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. Antimicrob Agents Chemother 2001; 45(3): 723-726.

42. Povilonis J, Šeputienė V, Ružauskas M, et al. Transferable class 1 and 2 integrons in Escherichia coli and Salmonella enterica isolates of human and animal origin in Lithuania. Foodborne Pathog Dis 2010; 7(10): 1185-1192.

43. Ahmed AM, Shimamoto T, Shimamoto T. Molecular characterization of multidrug-resistant avian pathogenic Escherichia coli isolated from septicemic broilers. Int J Med Microbiol 2013; 303(8): 475-483.

44. Kheiri R, Akhtari L. Antimicrobial resistance and integron gene cassette arrays in commensal Escherichia coli from human and animal sources in IRL. Gut Pathog 2016; 8(1): 40. doi: 10.1186/s13099-016-0123-3.

45. Piccirillo A, Giovanardi D, Dotto G, et al. Antimicrobial resistance and class 1 and 2 integrons in Escherichia coli from meat turkeys in Northern Italy. Avian Pathol 2014; 43(5): 396-405.

46. Bass L, Liebert CA, Lee MD, et al. Incidence and characterization of integrons, genetic elements mediating multiple-drug resistance, in avian Escherichia coli. Antimicrob Agents Chemother 1999; 43(12): 2925-2929.

47. Oosterik LH, Peeters L, Mutuku I, et al. Susceptibility of avian pathogenic Escherichia coli from laying hens in Belgium to antibiotics and disinfectants and integron prevalence. Avian Dis 2014; 58(2): 271-278.

48. Soufi L, Sáenz Y, Vinué L, et al. Escherichia coli of poultry food origin as reservoir of sulphonamide resistance genes and integrons. Int J Food Microbiol 2011; 144(3): 497-502.

49. Cocchi S, Grasselli E, Gutacker, et al. Distribution and characterization of integrons in Escherichia coli strains of animal and human origin. FEMS Immunol Med Microbiol 2007; 50(1): 126-132.