Changes in antimicrobial resistance patterns and dominance of extended spectrum \(\beta\)-lactamase genes among faecal \textit{Escherichia coli} isolates from broilers and workers during two rearing periods

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\textbf{ABSTRACT}

The emergence of antibiotic-resistant \textit{Escherichia coli}, especially extended-spectrum \(\beta\)-lactamase (ESBL)-producing strains, in the intestinal tract of broilers could be a threat to poultry and human. We investigated changes of antimicrobial resistance patterns and frequency of ESBL genes among faecal \textit{E. coli} isolates of broilers and workers in five different farms during two rearing periods in Iran. In this regard, \textit{E. coli} was isolated from rectal swabs of the workers and cloacal swabs of the broilers. After detection of antibiotic resistance patterns, phenotypic and genotypic characterisation of ESBL phenotype in these strains, carriage of the resistance genes on their crude plasmid extracts and diversity of plasmid profiles were analysed. Accordingly, multidrug-resistant (MDR) patterns were detected in a high percentage of \textit{E. coli} strains from the workers (72.7%) and poultry (92.3%). ESBL-producing \textit{E. coli} strains were identified in these farms throughout the two periods of rearing (6.3%). \textit{bla}_{\text{CTX-M-1}}, \textit{bla}_{\text{CTX-M-61}}, \textit{bla}_{\text{TEM-16}} and \textit{bla}_{\text{TEM-1}} were characterised in 6 (Period I/II: 4/2), 1 (Period I), 2 (Period II) and 9 (Period I/II: 5/4) strains, respectively. The first isolation of \textit{E. coli} strains harbouring the \textit{bla}_{\text{TEM-16}} gene in chicken is reported in this study. In conclusion, results of this study showed that chickens could serve as a reservoir for ESBL-producing \textit{E. coli} strains. These strains could carry clinically important ESBL or new emerging \(\beta\)-lactamases genes. Early colonisation and selection of the resistant strains during rearing periods proposed illegal use of antimicrobials as the cause of change in resistance patterns in the studied farms.

\textbf{Introduction}

\textit{Escherichia coli}, as part of the intestinal microbiota, is one of the most important and common bacterial agents responsible for different intestinal or extra-intestinal infections in human and animal populations (Miles et al. 2006). These infections are treated by common antibiotics; however, treatment failure can be caused, which result in increased treatment costs and mortality (Nunan and Young 2012). Uncontrolled prescription of antibiotics in veterinary medicine provides a selective pressure for emergence of resistance \textit{E. coli} strains not only among its known pathotypes, but also among the commensal strains (Miles et al. 2006). Antibiotics are used in food animals for control of bacterial infections and are continuously fed to their foods as antimicrobial growth promoters. Because of their roles in dissemination of resistance gene markers in the community, the existence of the resistant commensal strains in the gastrointestinal tract of food animals acts as a threat to public health (Nunan and Young 2012). Integrated surveillance study is necessary to track their development in food animals and their products.

The broilers intestinal tract can colonise with \textit{E. coli} strains presenting resistance phenotypes related to extended spectrum \(\beta\)-lactamases (ESBLs), which their emergence is a cause of concern in countries with...
uncontrolled usage of β-lactams in their farms (Engberg et al. 2000; Diarrassouba et al. 2007; Karczmarczyk et al. 2011). These strains may develop temporary during the breeding or growth, spread among the commensal strains through transmissible genetic elements, and persist in a poultry house long time. Workers of poultry house who are in direct contact with these strains are at highest risk of contamination. There are several reports that support the transfer of resistance genes between E. coli strains from animals and humans. Although some studies rejected this association, transmission of resistant E. coli strains and their plasmids from avian to human community was established in a study in Netherlands (van den Bogaard et al. 2001). In other studies, the colonisation of the human intestinal tract with resistant E. coli strains from chicken and spread of their antibiotic resistance plasmids were described (Cooke et al. 1971; Levy et al. 1976). Caya et al. (1999) and Kariuki et al. (1997) refused this association through comparing the phenotypes and genotypes of E. coli isolates from chickens and humans. To establish likelihood of this association, changes of resistance patterns along different time scales should be monitored in defined human and animal populations.

In the current study, possible changes of antimicrobial susceptibility patterns of faecal E. coli isolates and emergence of the ESBL-producing strains were investigated during the first week following entry of the chickens to the farms and the last week of a rearing period before slaughtering in five different poultry houses. The results were compared with the workers faecal isolates in the same farms, similarly.

Materials and methods

Bacterial isolates

A total of 471 cloacal swab samples from broilers and 24 rectal swabs from workers of five different poultry houses were collected over a six-month period in 2013 in Tehran, Iran. To study changes of the colonising intestinal E. coli during the breeding, we obtained the samples at two separate periods of growing up. The sampling from chickens less than a week comprised period I and samples taken from broilers a few days before slaughtering (6 weeks olds chicken) were considered as period II. The exact time of sampling and the number of poultry samples collected during each rearing period are shown in Supplementary material (Table A). The samples were collected from different broilers during the rearing periods. The sampling of workers was conducted in two periods to coincide with poultry. All the samples were transported to laboratory in the Cary–Blair medium (Merck, Germany). For isolation of E. coli, the swabs were immediately inoculated onto MacConkey agar medium (Merck, Germany). The plates were incubated for 18–24 h at 37°C. Single suspected colonies of E. coli were obtained from each sample for further analysis. Characterization of the suspected colonies was performed according to conventional laboratory biochemical tests (Odonkor and Ampofo 2013). All the characterised strains were stocked at −20°C. Ethical approval was obtained from the ethics committee at the Faculty of Veterinary Medicine, University of Tehran (Code 615-T) and for human samples from Shahid Beheshti University of Medical Sciences, Tehran, Iran (RIGLD).

Antibiotic resistance profiles

Antimicrobial susceptibility testing was done by the standard disk diffusion assay (Kirby- Bauer method) on Mueller–Hinton agar plates (Merck, Germany), using overnight cultures at a 0.5 McFarland standard followed by incubation at 37°C for 16 to 18 h. Interpretation was done according to the latest CLSI guidelines (CLSI 2012). Antibiotic susceptibility was studied against tetracycline (30 μg), ciprofloxacin (5 μg), chloramphenicol (30 μg), ampicillin (10 μg), gentamicine (10 μg), cefepime (30 μg), ceftazidime (30 μg), cefhalotin (30 μg), cefalexin (30 μg), cefoxitin (30 μg), ceftriaxon (30 μg) and meropenem (10 μg) (PadtanTeb, Iran). Resistance phenotypes related to ESBLs were detected by performing confirmatory tests in the cases of the resistant isolates to 1st-, 2nd- and 3rd-generation cephalosporins. For this purpose, ceftazidime (30 μg) and ceftazidime–clavulanic acid disks (30–10 μg; Rosco, Denmark) were used (Rawat and Nair 2010). E. coli ATCC 10559 was used as reference strain in all the experiments. Multidrug-resistance (MDR) and pandrug resistance (PDR) phenotypes were defined based on Magiorakos et al. (2012) guideline for the members of Enterobacteriaceae. MDR E. coli was defined as non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos et al. 2012).

Plasmid profiling

Correlation of the resistance patterns with plasmid profiles of the E. coli strains was determined in strains presenting ESBL drug resistance phenotype. The plasmid extracts were prepared using the alkaline lysis
method (Sambrook and Russell 2001). Counts and patterns of the plasmids were analysed in 0.8% agarose gel after electrophoresis and staining under ultraviolet light using an ultraviolet transilluminator.

**PCR amplification and sequencing of ESBLs genes in Escherichia coli strains**

The presence of bla TEM, bla SHV and bla CTX-M genes was investigated by polymerase chain reaction (PCR) on plasmid extracts of the ESBL-producing strains (Hu et al. 2006). PCR primer sequences and conditions used in this study were shown in Table 1. All PCR amplicons were analysed by gel electrophoresis on 1.2% (w/v) agarose gel and stained with ethidium bromide (0.5 mg/ml). To verify the detected genes in E. coli strains and characterise their types, related PCR products were subjected to bidirectional sequencing (Viragene Co., Iran) using the same primers used for their amplification. The nucleotide sequences obtained from various isolates were assembled and aligned with GenBank reference sequences using the ClustalW application. The sequences were submitted to GenBank and accession numbers were obtained. Translated amino acid sequences were compared with published sequences in the Lahey organisation (http://www.lahay.org/studies) and types of the ESBLs were determined based on defined amino acid changes.

**Statistical analysis**

Significant relationship between the resistance patterns of avian E. coli and workers’ strains was investigated using GraphPad Quick calcs (GraphPad Software Inc., La Jolla, CA, USA). NTSYS software (version 2.0) was used for comparison of phenetic data (resistance patterns) of the strains in each and among separate poultry houses.

**Results**

**Phenotypic characterisation of antimicrobial resistance**

Out of the 495 collected samples obtained from farms A-D (located in the East of Tehran) and farm E (located in the south-east of Tehran), nearly 94% of the samples showed positive culture results for E. coli (workers, 91.6% [22/24] and poultry farms, 94% [444/471]). Most of the isolates was related to lactose-positive E. coli strains (98.7%). Different rates of resistance to antibiotics were detected. Table 2 represents diversity and frequency of antimicrobial resistance among the E. coli strains in the poultry and workers. Related MDR patterns of the workers and poultry strains that were detected in the two growth periods were shown in Table 3. Nearly, 3.2% (15/466) and 0.4% (2/466) of the strains showed possible extensively drug-resistant (XDR) and possible pandrug-resistant (PDR) phenotypes, respectively. E. coli strains that were isolated from the second period showed a significant increase in the frequency of 5MDR pattern (p value = .007), while the frequency of 3MDR pattern was significantly decreased (p value = .004). The strains depicted triple to septate MDR patterns in both periods of growing up in all the poultry houses. The strains with eight and nine MDR patterns were detected only in the second period of growing up in the poultry houses C, D and E. These results showed high frequency of MDR patterns in the workers (72.7%; 16/22) and the broilers (92.5%; 411/444). While, triple and quadruple drug resistance patterns were mainly observed among the workers’ strains, resistance to higher numbers of the antibiotic categories (five to nine antibiotics) was detected in the avian strains (Table 2). The emergence of new resistance patterns was also detected in the second rearing period in all the studied farms.

As was shown in Figure 1, resistance to tetracycline (90.8%) and ceftazidime (0.2%) was detected as the highest and lowest rates among the tested
antibiotics. Analysis of the results for these farms showed no significant difference in the rates of resistance to antibiotics tetracycline (76.1–96.2%), ampicillin (60–88.7%), chloramphenicol (56.5–87.5%), and ciprofloxacin (51–74.2%). However, in the cases of antibiotics, cephalaxin (50% vs. 1.4–20.8%), cephalothin (52.2% vs. 1.4–20.8%), cefoxitin (50% vs. 1.4–20.8%) and ceftriaxone (47.8% vs. 1.4–20.8%), these analyses showed significant increases in rates of the resistance in farm B compared to the other farms (p value <0.001). Resistance to gentamicin, as an aminoglycoside, was determined as 30.4%. Resistance to meropenem was only detected among the strains that were isolated from farm E (68.6%). In contrast to farms A, C and E, farms B and D showed increased rates of resistance to 1st, 2nd, and 3rd generation cephalosporins. Among the workers’ strains, rates of resistance to meropenem, chloramphenicol, ciprofloxacin, ampicillin, ceftriaxone, and ceftazidim showed significant differences compared with those from the poultry. Although most of the workers’ strains presented lower percentages of resistance to these antibiotics, higher rates of resistance to meropenem and ampicillin were observed in these strains. Analysis of the resistance patterns between the poultry strains, which were isolated during the first (Period I, one-week old) and second (Period II, over 6-week old) rearing periods, showed increased rates of resistance to most antimicrobial families, including penicillins, tetracyclines, phenicols, aminoglycosides, and quinolones. TE + PEN + PHE + FQ was the most common MDR pattern in the poultry strains, which also detected in the workers’ (Table 3). Changes in resistance patterns of the isolates between the two study periods were summarised in Table 3.

Table 2. Prevalence of drug resistance patterns in avian and workers E. coli isolates in two different growth periods.

| Resistance to different classes of Antimicrobials | Avian isolates N (%) | Workers isolates N (%) |
|---------------------------------------------------|----------------------|------------------------|
|                                                   | Period I* (N = 174)  | Period II* (N = 270)   |
|                                                   | Period I/Period II   | p value                |
|                                                   |  |                       |
| 0                                                 | 5 (2.9)              | 2 (0.7)                | 0/0 | 0/0 | 2 (0.4) |
| 1DR                                               | 13 (7.5)             | 9 (3.3)                | 0/0 | 0/0 | 1 (7.7) |
| 2DR                                               | 5 (2.9)              | 3 (1.1)                | 0/0 | 0/0 | 1 (7.7) |
| 3DR                                               | 31 (17.8)            | 12 (4.4)               | 0/0 | 0/0 | 1 (7.7) |
| 4DR                                               | 28 (16)              | 84 (31.1)              | 2/0 | 0/0 | 79 (16.2) |
| 5DR                                               | 24 (13.8)            | 55 (20.4)              | 3/5 | 0/0 | 113 (23.1) |
| 7DR                                               | 31 (15.5)            | 31 (11.5)              | 3/6 | 0/0 | 79 (16.2) |
| 8DR                                               | 1 (0.6)              | 14 (5.2)               | 1/8 | 0/0 | 15 (3.1) |
| >9DR                                               | 0                    | 2 (0.7)                | 0/0 | 0/0 | 2 (0.4) |

aPeriod I: E. coli isolates from chickens less than 7-day old; Period II: E. coli isolates from chickens more than 45-day-old. 1DR: Triple resistance patterns; 2DR: Quadruple resistance patterns; 3DR: Quintuple resistance patterns; 4DR: Sextuple resistance patterns; 5DR: Septuple resistance patterns; 7DR: Octuple resistance patterns; 8DR: Nonuple resistance patterns.

Detection of ESBL genetic determinants

ESBL-encoding E. coli strains were characterised in the broilers’ isolates (6.3%) in both two rearing periods (Period I, 4.6% [8/174] and Period II, 7.4%, [20/270]). This phenotype was not characterised in the workers’ samples. Farm D showed relatively higher contamination rate with the strains presenting ESBL phenotype (39.3%, 11/28) compared with the other ones, including farm B (21.4%, 6/28), farms A, C (14.3%, 4/28, in each case) and E (10.7%, 3/28). Resistance to five or greater antimicrobial categories was observed mainly among the strains presenting ESBL phenotype (Table 2). While 12 out of 28 E. coli strains that showed ESBL phenotype (42.8%) carried blaTEM or blaCTX-M transfer of these genetic determinants was not established among the remaining 16 strains. The characterised β-lactamases were included blaCTX-M-1, blaCTX-M-61, blaTEM-116 and blaTEM-1 detected in 6 (Period I/II: 4/2), 1 (Period I), 2 (Period II) and 9 (Period I/II: 5/4) strains, respectively. While co-carriage of blaCTX-M-1 or blaCTX-M-61 with blaTEM-1 was detected among six strains (Period I/II: 4/2), blaglyc was not detected in the studied E. coli strains in these flocks. Following accession numbers were obtained for these β-lactamases: KP308218.1, KP308219.1, KP634884, KP634886, KP634888, KP634894, KP634892, KP634895, KP634896, KP634889, KP634897, KP634899, KP634887, KP634898, KP634890, KP634885, KP634893, and KP634891.

Similarity of Plasmid profiles in ESBL-producing E. coli strains

The same plasmid patterns were detected among some of the ESBL-encoding strains in each poultry house that was different from those from other farms.
Table 3. Frequency of faecal *E. coli* isolates with different MDR patterns in Period I (I) and Period II (II) in 5 different farms (A, B, C, D, E) in Tehran, Iran.

| MDR patterns (a) | A (I) | B (I) | C (I) | D (I) | E (I) | Workers (I) | E (II) | B (II) | C (II) | D (II) | E (II) | Workers (II) | Total (II) | Total (I) |
|------------------|-------|-------|-------|-------|-------|-------------|-------|-------|-------|-------|-------|-------------|-----------|-----------|
| TET + PEN + PHE  | 13 (29.5) | 1 (2) | 0 | 0 | 0 | 1 (3.7) | 0 | 3 (4.2) | 0 | 0 | 0 | 18 (3.9) | 28 (6.3) | 819 |
| TET + PEN + FQ   | 2 (4.5) | 2 (3.9) | 0 | 2 (5.3) | 2 (10.6) | 0 | 2 (7.4) | 0 | 1 (1.4) | 2 (2) | 1 (7.7) | 0 | 14 (3) | 59 (13) |
| TET + PEN + PHE + CAR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 (2.8) | 3 (2.9) | 0 | 1 (11.1) | 6 (1.3) |
| TET + PEN + PHE + FQ | 18 (40.9) | 25 (47) | 0 | 12 (31.6) | 11 (57.7) | 6 (14.4) | 12 (44.4) | 8 (25.8) | 2 (2.8) | 2 (2) | 1 (7.7) | 2 (22.2) | 99 (22.1) |
| TET + PEN + PHE + AMI + FQ | 0 | 14 (27.4) | 0 | 3 (7.6) | 3 (15.8) | 20 (47.1) | 0 | 18 (50) | 3 (4.2) | 1 (1) | 0 | 62 (1.3) | 8 (1.7) |
| TET + PEN + PHE + FQ + CAR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (1.4) | 17 (16.7) | 0 | 0 | 18 (3.9) | 5 (1.1) |
| TET + PEN + PHE + CMY + CAR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 (5.6) | 4 (3.9) | 0 | 0 | 8 (1.7) | 2 (0.4) |
| TET + PEN + FQ + CMY + CAR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 (4.2) | 2 (2) | 0 | 0 | 5 (1.1) | 2 (0.4) |
| TET + PEN + FQ + NE-CEPH + CMY + E-CEPH | 0 | 0 | 0 | 4 (8.4) | 1 (5.3) | 0 | 0 | 0 | 2 (2.8) | 0 | 0 | 6 (1.3) | 10 (2.2) |
| TET + PEN + PHE + NE-CEPH + CMY + E-CEPH | 0 | 11 (78.5) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 (2.3) | 2 (0.4) |
| TET + PEN + PHE + NE-CEPH + CMY + CAR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 (7) | 28 (27.5) | 0 | 0 | 33 (7.1) | 3 (0.6) |
| TET + PEN + PHE + FQ + NE-CEPH + CMY + E-CEPH | 0 | 4 (7.9) | 2 (14.3) | 3 (7.6) | 1 (5.3) | 2 (4.8) | 3 (11.1) | 1 (3.8) | 0 | 0 | 0 | 16 (3.5) | 8 (1.7) |
| TET + PEN + PHE + FQ + NE-CEPH + CMY + CAR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (1.4) | 6 (5.9) | 0 | 0 | 7 (1.5) | 3 (0.6) |
| TET + PEN + FQ + NE-CEPH + CMY + E-CEPH | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 18 (23.9) | 12 (11.8) | 1 (7.7) | 0 | 31 (6.6) | 5 (1.1) |
| Other MDR patterns (b,c) | 0 | 0 | 1 (2.3) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 (2.1) | 5 (1.1) |
| Total | 38 (80.4) | 51 (98.2) | 14 (100) | 33 (94) | 19 (100) | 42 (100) | 21 (77.7) | 33 (99.9) | 67 (91.1) | 96 (95.6) | 7 (53.9) | 9 (99.9) | 430 (91.9) | 28 (6.3) |

(a) MDR definition: non-susceptibility to at least one agent from three or more antimicrobial categories; Abbreviation of drug families: AMI: Aminoglycosides; CAR: Carbapenems; CMY: Cephamycins; E-CEPH: extended-spectrum cephalosporins; FQ: Fluoroquinolones; NE-CEPH: non-extended spectrum cephalosporins; TE: Tetracyclines; PEN: Penicillins; PHE: Phenicol.

(b) Period I.

(c) Period II.

Other MDR patterns: Resistance patterns with frequency lower than 0.2%.

The frequency of ESBL phenotype was estimated among all the strains, including those that were collected from the poultry and workers samples. This phenotype was detected among the poultry strains in a frequency of 6.3%.
Gel electrophoresis of the plasmids showed carriage of low- and high-molecular weight plasmids in these strains (Figure A, Supplementary material). Similarity of the plasmid patterns was observed among the strains in farms C and D, individually. Comparison of the plasmid patterns among the strains collected from different poultry houses showed a similar pattern in two strains isolated from farms C and D; however, the pattern was not identical.

Comparison of antimicrobial resistance patterns

Biotyping of the strains subjected to antibiotic susceptibility testing was performed according to their resistance profiles based on a distance cut-off point of 100%. *E. coli* strains of farm E, which was located in the south-east of Tehran showed greatest diversity of resistance patterns compared with the other farms located in the East of Tehran. The most similar resistance patterns were observed among the strains collected from farms A and C. Analysis of the obtained clusters in all the poultry houses showed a correlation between the resistance patterns and the sampling time. Accordingly, strains that were obtained from Period I or II of the rearing were mostly clustered with the other strains from the same sampling period in other farms. This similarity was also observed among the collected strains from each poultry house (Figure B, Supplementary material). However, coexistence of the strains obtained from different sampling times was observed in few clusters. Similarity of the resistance patterns between the workers and chickens strains was detected in 5 out of the 22 workers’ strains, which was not statistically significant. Cross contamination of chickens and workers was postulated just in one poultry house (farm E), so that the strains were imipenem resistant and showed similar resistance patterns (Figure B, Supplementary material).

Discussion

Surveillance studies could provide valuable information about antimicrobial resistance rates in *E. coli* strains and risk of their transmission. In this study, we examined resistance patterns of collected *E. coli* strains from faecal samples of broiler chickens and workers
related to their farms. The strains were highly resistance to tetracycline, ampicillin, chloramphenicol and ciprofloxacin. This finding is consistent with a previous study from Iran that reported increased rates of resistance to tetracycline and chloramphenicol among the strains isolated from the broilers (Rafiei Tabatabaei and Nasirian 2003). The use of oxytetracycline (a member of tetracyclines) as a feed additive for growth promotion and prevention of diseases is common in the broiler rearing in Iran (Salehzadeh et al. 2006). Therefore, cross-resistance due to the use of this agent possibility was the cause of increase in the resistance to tetracycline in the studied farms. Usage of fluoroquinolones in veterinary medicine is considered as a concern for the treatment of serious infections in human medicine (Van et al. 2008). Administration of Fluoroquinolones (enrofloxacine) in the studied farms was postulated according to our results, since significant increase in the rate of resistance to ciprofloxacin was observed at second period of rearing. Resistance to ciprofloxacin in our strains was higher than those reported from European countries (Boerlin et al. 2005). In the case of chloramphenicol, despite its banned use in Iran, high rates of resistance to this antibiotic were detected in all the poultry houses. The presence of chloramphenicol resistance phenotype in E. coli strains was reported by earlier studies in Iran (White et al. 2000). The high rate of resistance could be explained by the usage of florfenicol in these farms. Resistance to this antibiotic is in accordance with resistance to chloramphenicol, which is mediated by a plasmid encoded gene, flo (Keyes et al. 2000). In a report from Pan-European monitoring surveillance study, 13.3% of the chicken strains showed resistance to this antibiotic, which was higher than those reported from United States of America (0.6%). Selection of the resistant strains with unrelated antimicrobials was proposed in this report to explain higher level of resistance to this antibiotic in the E. coli strains in Europe (Abbott et al. 2011).

Similar to our results, increase in the numbers of resistance phenotypes during the growth of the chickens and change in faecal microbiome was established by Ozaki et al. (2011). These authors showed that the change can occur even in the absence of antimicrobial administration.

The lowest rate of resistance was related to broad spectrum cephalosporins in the studied poultry houses (0.2–12%). This finding was in accordance with those reported by de Jong et al. (2012) in Europe. The increased administration of extended-spectrum β-lactams in food animals over the last two decades resulted in the emergence of resistance strains to these antibiotics (Szmolka and Nagy 2013). While low rate of the strains presenting ESBL phenotype was detected in our study (6.3%), its presence is a threat in these farms. To the best of our knowledge, this is the first report that described this emergence in Iran. Girlich et al. (2007) detected a frequency of 10.7% ESBL-encoding E. coli strains from healthy poultry in France in 2005 and suggested food animals as a possible reservoir for ESBL genes. Colonization of ESBL-producing bacteria in the gastrointestinal tract of food animals may contribute to the increased incidence of infection with these bacteria in human populations (Hall et al. 2011). However, this correlation was not the case in our study, since no E. coli strains presenting ESBL phenotype was isolated from faecal samples of the workers. Our findings demonstrated blaCTX-M-1 as the most common type of ESBLs in the chicken isolates. This beta lactamase was detected as the most common blaESBL genes among Enterobacteriacea in Europe and Asia (Bonnet 2004). Local studies in Iran reported CTX-M as the most dominant ESBL in E. coli strains from clinical samples (Ramazanzadeh 2010). Mirzaee et al. (2009) reported dominance of blaCTX-M group 1 as common CTX-M β-lactamase genes among E. coli strains isolated from hospitalised patients in Iran, which was similar to those reported in the Switzerland, France and Austria. CTX-M-61 is rarely found in Enterobacteriacea and its detection in E. coli strains is scant. The presence of blaCTX-M-1/61 in poultry and farm workers was proposed in a recent study in Germany. However, differentiaotin of these two enzymes was not verified (Dahms et al. 2015). The first isolation of TEM-116 ESBL-encoding strains in chicken was referred to a Shigella flexneri isolate in China (Hu et al. 2008). Isolation of E. coli strains carrying blaTEM-116 was also described in clinical samples (Fernandes et al. 2014); however, according to our knowledge, there is no report about its isolation from the chicken. The absence of the examined ESBL-encoding genes among the remaining E. coli strains that presented ESBL resistance phenotype, proposed the presence of other ESBLs genes, including blaPER, blaVEB, blaSEL-1, blaGES-1, blaSFO-1, blaTLA-1, and blaTLA-2, which needs further studies.

In our study, the observed resistance to meropenem was surprising, although the rate was low similar to the other reports from European, American and Asian countries (Sosa et al. 2010; Aly et al. 2012; Cho et al. 2012). There are no previous reports of the presence of this resistance phenotype in E. coli strains from poultry in Iran. In the current study, resistance to meropenem was only detected in farm E, which was in association to its presence in the workers faecal samples in this farm. This farm was located in distinct
geographic location where other farms were placed. This difference shows the importance of the control on all animal food production sectors. Analysis of the antibiotic susceptibility patterns showed the same resistance phenotype for the imipenem resistant strains.

Resistance to multiple antibiotic families was detected in the studied farms in Iran. In the Europe, lower rate of MDR patterns (31.1%) was reported by the European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) in E. coli strains from broilers in 2012 (EFSA 2014). However, most of the strains in our study showed multi-drug resistant patterns (87.7%, Period I and 95%, Period II) that were similar to those reported previously (Van et al. 2008). Although, results of our study showed influence of antimicrobials on selection of the resistant E. coli strains, isolation of the MDR strains in Period I of their rearing proposed their early contamination in related hatcheries. As was shown by Torok et al. (2011), the use of antimicrobials in hatcheries promotes the selection of resistance strains during the first week of life (≤7 days) in both broilers and layers. In addition to the increased consumption of antibiotics, overcrowding and poor sanitation seems to be the possible reason for developing and spreading of the MDR strains in the studied poultry houses. The most frequent MDR pattern in these farms was TE + PEN + PHE + FQ, which was in accordance with the most common MDR patterns in Europe (TE + PEN + FQ) and America (TE + PEN + PHE). These MDR patterns were also reported by another study (Tadesse et al. 2012). Transmission of the resistant strains between the poultry and farmers was not confirmed in this study, since different patterns of antibiotic resistance (except farm E) were characterised in the workers compared with the broiler chickens during the two rearing periods. However, as a main limitation, in this study, we just analysed the workers who were in direct contact with the chickens in each farm. To find a better relationship, workers of slaughterhouses and consumers in the community also need to be followed. Dissemination of E. coli strains from poultry to farmers was established in a study in the Netherlands (van den Bogaard et al. 2001). These strains conferred high rate of resistance to amoxicillin and tetracycline and showed the same pulsotyple. The observed changes of MDR patterns between the two rearing periods and the emergence of possible PDR strains (resistance to nine antimicrobial families) in current study was similarly determined in a study by da Costa et al. (2009). They explained this shift of resistance profile by capacity of E. coli strains for genetic exchange of resistance genes and change in their community structure under the selective pressure of antibiotics. Existence of various plasmids was revealed in these strains by plasmid profiling, which proposed their association with the acquisition of new resistance phenotypes. This association was supported by the detection of the same plasmid profiles among the strains collected from each poultry house. However, no similar plasmid pattern was observed in the strains of distinct farms. Horizontal transmission of the same plasmid harbouring an ESBL gene among different E. coli strains was indicated by this study, which proposed the existence of a common source of transmission in some studied poultry houses. This transmission could result in some selective conditions, since most of the strains that conferred ESBL phenotype were obtained from the second study period. More investigation is needed to establish this association in these farms.

**Conclusions**

In conclusion, results of the present study highlighted the emergence and enrichment of MDR phenotypes among E. coli isolates in the gut of healthy broiler chickens and workers of different poultry houses during the rearing periods in Iran. Resistance to five and greater antimicrobial families in these flocks was mainly associated with infection of ESBL-producing strains (bla\textsubscript{CTX-M-1}, bla\textsubscript{CTX-M-61}, bla\textsubscript{TEM-116}). Similarity of plasmid profiles among the ESBL strains in each farm and carriage of the genes on plasmid extracts, proposed plasmids as main vehicle of these genetic elements among these strains. While comparison of resistance phenotypes verified correlation of E. coli strains between the broilers and workers in one farm, administration of antibiotics, especially Fluoroquinolones and Phenics, was postulated as main reason for changes of resistance phenotypes in these farms at the second rearing period. Programs for monitoring antimicrobial use in hatcheries and poultry houses are necessary to control the emergence of resistant E. coli strains in broilers’ farms and prevent their transmission to human population.

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**Disclosure statement**

The authors declare that they have no conflict of interest.
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