ORIGINAL ARTICLE

Phenotypic and genotypic detection of ESBL-producing *E. coli* isolates from chicken skin in Bosnia and Herzegovina

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ABSTRACT

Aim To identify *E. coli* from chicken meat, establish their antibiotic resistance profiles and to confirm ESBL isolates with real time PCR, as well as to identify risk factors and farming practice associated with the antimicrobial resistance *E. coli*.

Methods The study included 100 chicken skin samples collected randomly from retail supermarkets, butcheries and slaughterhouses. Disk susceptibility testing was performed using the Kirby-Bauer method. Detection of ESBL-producing isolates was performed with double disk synergy test. Molecular analysis of phenotypic ESBL-producing *Escherichia coli* strains was performed at 7500 real time PCR System. Molecular-genetic analysis included detection of CTX-M 1, 2, and 9 gene families and mutations in the TEM and SHV encoding extended spectrum β-lactamases.

Results Prevalence of the phenotypic ESBL-producing *E. coli* isolates was 29%, and they exhibited remarkable sensitivity to carbapenems (100%) as well as to amikacin (93.10%). All ESBL-producing strains were multidrug resistant. Molecular analysis was performed as the final confirmation of the production of extended spectrum β – lactamases for 24 isolates out of 29 phenotypicaly ESBL-producing *E. coli* isolates.

Conclusion It is important to pay attention to people's awareness of bacterial antimicrobial resistance in food chain, as well as to understand its effects on human health and the environment. Phenotypic and molecular analysis demonstrated the presence of ESBL-producing *E. coli* isolates from chicken skin samples.

Key words: antimicrobial resistance, food safety, veterinary medicine

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Original submission:
15 May 2020;
Revised submission:
01 June 2020;
Accepted:
16 June 2020
doi: 10.17392/1206-20

Med Glas (Zenica) 2020; 17(2): 308-315

INTRODUCTION

Antimicrobial resistance is a complex and multifaceted problem that threatens human, animal health, the global economy, national and global security (1). The widespread use of antimicrobials in farming practices has been attributed to the presence of antimicrobial resistant bacteria in food (2,3). Currently, few data are available regarding the contamination of retail foods with *E. coli*, especially those that are multi-resistant and pathogenic (4).

Little is known about the antibiotic-resistant frequency of microorganisms in poultry that were raised by free-range farming, but it is known that poultry industries use antibiotics for therapeutic purposes and for growth promotion (5,6). An important issue in the context of increasing consumption and production of poultry meat is to ensure microbial safety (7).

In the community and environment poultry are recognized as an important source for dissemination of antimicrobial resistant *E. coli* (8).

Both extended spectrum beta-lactamase (ESBL) and carbapenemase, which are produced mainly in *Escherichia coli* are utilized as crucial resistance mechanism to cephalosporins and carbapenems (9).

Pathogenic *E. coli* in poultry represent a direct threat to both poultry industry and human health as they may result in hard-to-treat infections (10). Farm animals, especially broiler chickens, affected by ESBL-producing *E. coli*, can be transmitted via food chain from animals to humans (11,12).

Direct contact with broilers has been identified as a risk factor for carriage of extended-spectrum β-lactamase (ESBL) and plasmidic AmpC (pAmpC) producing *E. coli* for humans on broiler farms (13). In addition, resistant *E. coli* may act as transporters for antimicrobial resistant genes to other pathogens (13). Other pathogens may receive antimicrobial resistant genes from resistant *E. coli* who act as a transporter (14).

Administration of antimicrobial agents in developed countries is not only restricted for the treatment purpose, but they can also be used to enhance animal productivity, growth rate and feed conversion rate in food producing animals (15). Favourable conditions for persistence and spread strains with genetic traits that can resist to antimicrobials allow this type of farming (16).

Furthermore, *E. coli* encoding for ESBL and carabepenemase are resistant to more than one class of antimicrobials and hence they are multidrug resistant (MDR), presenting a serious challenge in healthcare settings (17). Enterobacteriaceae that produce ESBLs are a challenging problem in human and veterinary medicine due to the limitation in the treatment options (18).

The aim of this study was to isolate and identify *E. coli* from chicken meat, establish their antibiotic resistance profiles and to confirm ESBL isolates with real time PCR, as well as to identify risk factors and farming practice associated with the antimicrobial resistance *E. coli*.

MATERIALS AND METHODS

Materials and study design

This investigation was prospective, experimental and applied. An experimental part was conducted in the period February 2018 to February 2019 at the Institute of Biomedical Diagnostic and Research ‘’Genom“ Travnik and the Institute for Biomedical Diagnostics and Research NALAZ, Sarajevo.

This study was approved by the Doctoral Studies Council of the University of Tuzla, Faculty of Science and the Ethical Committee.

Methods

Isolation of *E. coli* from meat samples

Collection and preparation of samples, isolation of *Escherichia coli* from chicken skin (neck), as well as phenotypic detection of ESBL strains were performed in the Laboratory for Microbiology of the Institute for Biomedical Diagnostics and Research, GENOM Travnik. The study included 100 chicken skin samples (50 frozen samples and 50 raw samples), collected randomly from retail supermarkets, butcheries and slaughterhouses in Travnik, Bosnia and Herzegovina (Table 1). The samples were aseptically collected and then packaged in sterile polythene zip bags and carried to the laboratory in aseptic conditions in a cold box within two hours from the time of purchase. Duplicate samples were obtained whenever possible. All samples were analysed within 2-4 hours after their arrival to the laboratory. A sharp sterile knife was used to cut sample from surface in sterile tray. To isolate bacteria, a 25-g portion of sample

was placed into sterile 225 mL buffered peptone water. For the preparation of samples, the study used guidelines given in the Microbiology of Food and Feeding Standards - Preparation of test samples, initial suspensions and decimal dilutions for microbiological tests (19).

After the preparation of the samples, the microbiological analysis was carried out according to the ISO standard (20).

Antibiotic susceptibility testing. Mueller-Hinton agar (HiMedia, India) was used to perform antibiotic susceptibility testing of all *E. coli* isolates using 14 antibiotic discs (Mast Group, UK) following Clinical Laboratory Standards Institute (CLSI) standard (2017) (21): ampicillin 10 μg (AMP), cephalexin (30 μg) (CFX); ceftazidime (30 μg) - CAZ, imipenem (10 μg) (IMIP), aztreonam (30 μg) (ATM), ciprofloxacin (5 μg) (CIP), amikacin (AK) (30 μg), amoxicillin (25 μg) (AMX), ceftriaxone (30 μg) (CRO), meropenem (MRP) (10 μg), nalidic acid (30 μg), amoxicillin with clavulanic acid (20 μg-10 μg) (AUG), cefpodoxime (10 μg) (CPD), cefotaxime (30 μg) (CTX). *E. coli* isolates were inoculated in nutrient broth and incubated at 35 +/- 2 ˚C for 16-18 h.

After antibiotic susceptibility testing of all *E. coli* isolates, strains that had a phenotypical indication for ESBL production were selected, and the ESBL confirmatory test according to CLSI (21) was performed.

Extended spectrum β-lactamase (ESBL) confirmatory test. The ESBL phenotype was confirmed by assessing the ability of clavulanic acid (10 μg) to inhibit the activity of cefotaxime (30 μg) and ceftazidime (30 μg) in a standard combination disk diffusion test: if the addition of clavulanic acid increased the zone of inhibition by ≥5 mm when compared to the drug alone, the isolate was defined as an ESBL-producer (21). *Klebsiella pneumonia*e ATCC 700603 (Liofilchem, Italy), and as an ESBL negative strain of *E. coli* ATCC 25922 (Microbiologics, USA) were used as control strains.

After ESBL confirmatory test a double disk synergy test was performed using the following antibiotic disks: amoxicillin/clavulanic acid (30 μg), aztreonam (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg) and cefpodoxime (10 μg) (Mast group, UK) (19). Briefly, inoculums were prepared in sterile saline solutions from grown cultures. When 0.5 McFarland turbidity value was obtained for each bacterial inoculum, they were inoculated onto Mueller Hinton Agar (Hi Media, India) by sterile cotton swab. Then, amoxicillin/clavulanic acid was placed at the centre and the others around it that were far from each other for 24 mm from centre to centre. Subsequently, they were incubated at 35 +/- 2ºC for 16-18 h. A clear extension or protrusion (synergistic effect) of the edge of the inhibition zone of any of the antibiotics toward the disk containing amoxicillin/clavulanic acid was interpreted as positive for ESBL production (Table 1).

The MAR index was determined for all of 29 phenotypic ESBL-producing *E. coli* strains. The results were read by measuring diameter of the inhibition zone according to CLSI (21). Therefore, the isolates for each antibiotic were characterized as: sensitive (S), intermediate (I) and resistant (R). For each phenotypically confirmed ESBL isolate, a multiple antibiotic resistance indexing (MAR) was determined according to the formula:

The MAR index was calculated so that the numerator contains the number of antibiotics to which the isolate is resistant, and the denominator shows the total number of antibiotics used during the research.

DNA isolation and identification. DNA isolation from bacterial cells was performed at the Institute for Biomedical Diagnostics and Research "*Nalaz*" in the Molecular Diagnostic Laboratory in Sarajevo according to a protocol (Institute for Applied Laboratory Analysis Ltd, 2007) (Genial, Germany) (22). After the isolation of DNA from bacterial cells, an identification and amplification procedure was immediately started. At the beginning of the analysis, the number of reactions was determined, a positive and a negative control (water) with an IC reagent were prepared, and solution A was stored at -20 ° C. According to the parameters described in the User Manual Check Point (2015) ligation reaction mix was prepared and ligation reaction process was performed (23).

Real time amplification. A novel, rapid real-time PCR for the detection of ESBL genes in phenotypic ESBL-producing *Escherichia coli* strains was used (Check-MDR ESBL PCR; CheckPoints) (23). The principle of the Check-Points Real-time PCR assay is based on specific reco-

Table 1. Distribution of *E. coli* and phenotypic ESBL-producing *E.coli* chicken skin isolates according to the source

Chicken skin sample source	Frozen (50) samples			Fresh (50) samples		
	No (%) of samples	No (%) of *E. coli*	No (%) of phenotypic ESBL-producing *E. coli*	No (%) of samples	No (%) of *E. coli*	No (%) of phenotypic ESBL-producing *E. coli*
Supermarket	22 (44)	12 (24)	5 (10)	24 (48)	20 (40)	8 (16)
Butcheries	18 (36)	8 (16)	1(2)	17 (34)	15 (30)	11 (22)
Slaughterhouse	10 (20)	4 (8)	0	11 (22)	5 (10)	4 (8)
Total	50	24	6	50	40	23

gnition of target sequences by two adjacent DNA probes, followed by ligation of these probes. Subsequently, the ligated probes were amplified by real-time PCR on an ABI 7500 PCR (Thermo Fisher Scientific, USA) instrument using universal primers and a molecular beacon. In a typical reaction, the PCR product was formed exponentially. Because it took several cycles to replicate enough DNA copies, the curve of the ratio of the fluorescence intensity to the number of cycles showed a sigmoidal shape. In late cycles, the substrates of the reaction (DNA, dinucleotides, enzyme) were depleted, the PCR product no longer doubles and the curves become flat - a "plateau". The point on the curve when the fluorescence intensity increases sharply, usually several standard deviations above the baseline, is called the threshold cycle (Ct value). Analyses and data interpretations were performed according to the manufacturer's instructions.

Molecular-genetic analysis included an examination of the presence of CTX-M 1, 2, and 9 gene families and mutations in the TEM and SHV encoding extended spectrum β-lactamases. For samples with FAM CT> 36 or undetermined, and with IC Cy5> 36 or undetermined, the analysis was not well performed and the DNA extraction of the bacteria needed to be repeated. Real time amplification results were read from the amplification panel (23).

Statistical analysis

Student's t-test was used for testing of parametric significance. T test was used to confirm whether there was a statistically significant difference at the $p<0.05$ between the inhibition zone sizes of used antibiotics, for phenotypically detected ESBL- and non-ESBL-producing *E. coli* isolates.

RESULTS

A total of 100 chicken skin samples were analysed, of which 50 were fresh and 50 were frozen meat. We determined 64 *E. coli* isolates, 24 (48%) frozen meat samples and 40 (80%) fresh meat samples (Table 1). *E. coli* was mostly detected in supermarket fresh meat, 12 (24%) and frozen meat 8 (16%).

Antimicrobial susceptibility test results

Tested *E. coli* strains showed 100% resistance to penicillines, nalidic acid and cephalexin (Table 2). With regard to the third-generation cephalosporins, 100% ceftriaxone resistance was found, while 96.55% were cefpodoxime resistant. Resistance to aztreonam was found in 79.31% strains.

The phenotypic ESBL-producing *E. coli* isolates exhibited 100% sensitivity to carbapenem and 93.10% to amikacin. Amoxicillin with clavulanic acid showed a sensitivity of 82.75% and intermediate-sensitivity of 6.89% in ESBL-positive strains. Greater inhibition zone of at least five mm around the disc of cefotaxime with clavulanic acid (CTXCV) than around the disc of cefotaxime (CTX) alone (93.1%), and ceftazidime disc with clavulanic acid (CAZCV) relative to ceftazidime (CV) alone (82.75%) was found, indicating the presence of extended β-lactamase (Table 3).

The average MAR index of phenotypic ESBL-producing *E. coli* strains was 0.70, maximum 0.78, and the minimum 0.57 (Table 3).

Statistically significant difference between the sizes of inhibition zones for antibiotics used

Table 2. Indication of the zone inhibition size of 29 phenotypically identified *E. coli* strains from chicken skin on antibiotics

Zone inhibition (mm)	Antibiotic													
	IMI	MRP	AMX	AMP	AK	NA	CIP	AUG	ATM	CTX	CAZ	CRO	CPD	CFX
Average	25.38	26.90	0.41	1.17	20.24	1.14	6.03	18.69	12.76	6.66	5.21	4.79	3.45	0.00
Maximum	30	30	7	10	24	12	26	26	27	27	24	20	17	0
Minimum	23	23	0	0	8	0	0	10	0	0	0	0	0	0

IMI, imipenem; MRP, meropenem; AMP, ampicilin; AMX, amoxicilin; AK, amikacin; NA, nalidixic acid; CIP, ciprofloxacin; AUG, amoxicillin-clavulanic acid; ATM, aztreonam; CFX, cefalexin; CTX, cefotaxime; CAZ, ceftazidime; CRO, ceftriaxone; CPD, cefpodoxime

Table 3. Antimicrobial profile of phenotypically confirmed ESBL *E. coli* isolates and multiple antibiotic resistance (MAR) index

Source of a strain	Sample tag	Antibiotic resistance profile								MAR INDEX
		CTX	CAZ	ATM	CRO	CPD	CAZ/CV	CTX/CV	Other antibiotics	
SM	Ch1FR	R	S	R	R	R	-	+	AMP, AMX, CFX, NA, CIP	0.643
SM	Ch2FR	R	S	S	R	R	-	+	AMP, AMX, CFX, NA, CIP	0.571
SM	Ch3FR	R	R	S	R	R	-	+	AMP, AMX, CFX, NA, CIP	0.643
SM	Ch4FR	R	R	R	R	R	+	+	AMP, AMX, CFX, NA, CIP	0.714
BT	Ch5FR	R	R	R	R	R	+	+	AMP, AMX, CFX, NA, CIP	0.714
SM	Ch6FR	R	R	R	R	R	+	+	AMP, AMX, CFX, NA, CIP	0.714
BT	Ch7F	R	R	S	R	R	+	+	AMX, AMP, CFX, NA	0.571
BT	Ch8F	R	R	R	R	R	+	+	AMX, AMP, CFX, NA,CIP	0.714
SM	Ch9F	R	R	S	R	R	+	+	AMX, AMP, CFX, NA, CIP, AK, AUG	0.786
SM	Ch10F	R	R	R	R	S	+	+	AMX, AMP, CFX, NA, CIP	0.643
SM	Ch11F	R	R	S	R	R	+	+	AMX, AMP, CFX, NA, CIP, AUG	0.714
BT	Ch12F	R	R	R	R	R	+	+	AMX, AMP, CFX, NA, CIP, AUG (I)	0.786
SM	Ch13F	R	R	R	R	R	+	+	AMX, AMP, CFX, CIP, NA, AK	0.786
SL	Ch14F	R	R	R	R	R	+	+	AMX, AMP, CFX, CIP, NA,	0.714
SL	Ch15F	R	R	R	R	R	+	+	AMX, AMP, AK, CIP, CFX, NA	0.786
BT	Ch16F	R	R	R	R	R	+	+	AMX, AMP, CIP,CFX, NA	0.714
SL	Ch17F	R	S	R	R	R	-	+	AMX, AMP, CIP, CFX, NA	0.643
BT	Ch18F	S	R	R	R	R	-	+	AMX, AMP, CIP, CFX, NA, AUG	0.786
SM	Ch19F	R	R	R	R	R	+	+	AMX, AMP, CIP, CFX, NA	0.714
BT	Ch20F	S	R	S	R	R	+	-	AMX, AMP, CIP, CFX, NA, AUG	0.643
BT	Ch21F	R	R	R	R	R	+	+	AMX, AMP, CIP, CFX, NA,	0.714
BT	Ch22F	R	R	R	R	R	+	+	AMX, AMP, CIP, CFX, NA, AUG	0.786
BT	Ch23F	R	R	R	R	R	+	+	AMX, AMP, CIP, CFX, NA, AUG(I)	0.786
SM	Ch25F	R	R	R	R	R	+	+	AMX, AMP. CIP, CFX, NA	0.714
BT	Ch36F	R	R	R	R	R	+	+	AMX,AMP, CIP(I), CFX, NA	0.714
SM	Ch26F	R	R	R	R	R	+	+	AMX, AMP, CIP, CFX, NA	0.714
SM	Ch27F	R	R	R	R	R	+	-	AMX, AMP, CIP, CFX, NA	0.714
BT	Ch28F	R	R	R	R	R	+	+	AMX, AMP, CIP, CFX, NA	0,714
SL	Ch29F	R	R	R	R	R	+	+	AMX, AMP, CFX, NA	0.643
Overall resistance rate		93.10	89.6	79.31	100	96.55	82.75 (+)	93.1 (+)	AMX, AMP, CFX, NA (100% R); CIP (89.65% R; 3.4% I); AUG (17.24% R; 6.89% I); AK (10.34% R)	

AMP, ampicilin; AMX, amoxicilin; CFX, cefalexin; AUG, amoxicillin-clavulanic acid; CAZ/CV, ceftazidim/clavulanic acic; CTX/CV, cefotaxim/clavulanic acid; CAZ, ceftazidime; CTX, cefotaxime; CPD, cefpodoxime; CRO, ceftriaxone; IMI, imipenem; MRP, meropenem; ATM, aztreonam; CIP, ciprofloxacin; NA, nalidixic acid; AK, amikacin; MAR, multiple antibiotic resistance; SM, supermarket; BT, butcheries; SL, slaughterhouse; F, fresh; FR, frozen; Ch, chicken; S, Sensitive; R, Resistant; I, Intermediate; +, positive ESBL test; -, negative ESBL test

between phenotypically ESBL-producing *E. coli* isolates and non-ESBL isolates was found for ceftazidim ($p<0.05$), ampicillin ($p<0.05$) and amoxicillin ($p<0.05$), but not with nalidic acid ($p=0.498$) and imipenem ($p=0.2$).

Molecular detection

After the detection of phenotypic ESBL-producing *Escherichia coli* isolates, the strains were subjected to molecular analysis. DNA isolation was derived from a total of 29 phenotypic ESBL-producing *Escherichia coli* strains.

Out of 29 analysed isolates, five did not have genes for extended spectrum β - lactamase production. It is clearly seen that the curves reached the amplification threshold of 0.05 over 40 cycles. Molecular analysis showed the presence of genes from the CTX family M-1, 2 and 9, as well as mutations in SHV and TEM in the total of 24 strains. *E. coli* produces broad spectrum β – lactamases (Table 4).

DISCUSSION

The World Health Organization (WHO) has included antimicrobial resistance as one of the top ten threats to global health in 2019 (24). There is no available scientific literature about the prevalence of ESBL strains of *E. coli* in foods of animal origin in Bosnia and Herzegovina. Considering recent studies (1-5, 11,12,14), it was necessary to carry out a study that would indicate the problem of antibacterial resistance as well as the occurrence of ESBL isolates of animal origin in our area, especially in chicken sources. In our study we detected 64% *E. coli* isolates from chicken skin samples. A similar prevalence of *E. coli*

Table 4. ESBL chicken skin *E. coli* strains confirmed by real time PCR analysis with Ct values

Sample tag	C_t^*
Ch5FR	22.82
Ch6FR	23.75
Ch7F	25.16
Ch8F	23.79
Ch9F	30.22
Ch10F	23.58
Ch11F	26.24
Ch12F	27.36
Ch13F	27.75
Ch14F	30.66
Ch15F	21.77
Ch16F	20.43
Ch17F	21.22
Ch18F	22.93
Ch19F	21.17
Ch23F	22.56
Ch21F	23.29
Ch26F	26.34
Ch1FR	22.89
Ch27F	23.51
Ch28F	28.51
Ch29F	20.26
Ch4FR	23.49
Ch3FR	21.13

*Ct value up to 31 was considered as positive result Ch, chicken; F, fresh; FR, frozen; Ct value (cycle threshold), number of cycles required for the fluorescent signal to cross the threshold; a relative measure of the concentration of target in the PCR reaction.

of 56.3% in poultry and beef meat samples was noted by Eyi & Arslan (25).

Overall prevalence of ESBL–producing *E.coli* isolates was 29% , with the resistance rate of 100% to penicillines, nalidic acid, cephalexin and ceftriaxon. None of the isolates were resistant to meropenem or imipenem.

Our study results are similar to the study of Davis et al. (26) which proved 87.6% overall prevalence of resistant *E. coli* isolates from chicken meat to 10 of 12 antibiotics tested. None of the isolates were resistant to amikacin or imipenem. These results are similar to the study results of Rahman et al. (27) who recovered 47.6% ESBL-producing *E.coli* isolates from chicken meat out of 42 samples. Also, a study conducted in Germany by Reich et al. (28) reports a high resistance of ESBL producing *Escherichia coli* from poultry to cefuroxime, cefotaxime, ceftazidime and ceftriaxone.

The value of the multiple resistance index (MAR) was assigned to determine the rate of antibiotic contamination of the analysed meat. According to different authors (29-31), a MAR index value greater than 0.2 indicates that the isolate originates from a high-risk source, e.g. from an environment where the antibiotic use is frequent. The obtained MAR index values in our study indicate that all isolates were from environments that are highly contaminated with antibiotics. In our work, minimum MAR index values were greater than 0.4 for all isolates. In the study of Hussain et al. (32), 78.5% of ESBL-producing *E. coli* was found to be multi-drug resistant.

Pacholewicz et al. (33) suggest that management factors like availability of adequate monitoring procedures and food handlers compliance with these procedures may influence the bacterial concentrations on carcasses. They demonstrated that compliance with procedures differed between slaughterhouses, and might be associated with faecal contamination of carcasses and thus with higher bacterial concentrations.

Molecular analysis of real time PCR was performed as the final confirmation of the production of extended spectrum β – lactamases for 24 of 29 *E.coli* isolates. Of 29 analysed isolates, five did not detect genes for extended spectrum β - lactamase production. It is clearly seen that the curves do reach the amplification threshold of 0.05 over 40 cycles. Molecular analysis showed the presence of genes from the CTX family - M-1, 2 and 9, as well as mutations in SHV and TEM in the total of 24 strains. The difference in the number of phenotypically and genotypically confirmed isolates can be explained by the limited PCR detection spectrum of the Check MDR ESBL kit used in the study. There are much more ESBL gene groups, so we assumed that the used kit did not detect other genes also responsible for ESBL production, explaining the difference in the number of phenotypically and genotypically confirmed ESBL strains.

Poultry – derived ESBL-producing *E. coli* isolates in the study of Hussain et al (32) harboured different variants of ESBL genes including $bla_{\text{CTX-M-15}}$ (40%), $bla_{\text{CTX-M-27}}$ (4%), $bla_{\text{CTX-M-55}}$, $bla_{\text{CTX-M-55}}$ (24%), $bla_{\text{TEM-1}}$ (16%) and $bla_{\text{SHV-122}}$ (14%).

Skin isolates are exposed to the environment, thus to a high diversity of micro-organisms, and have full access to the genetic pool presented by them. It is clear that chances of receiving resistance genes against antimicrobial agents are higher for skin isolates (34).

The increasing resistance of bacteria to the effects of antibiotic therapy is a major problem in both

veterinary and human medicine. Its solution requires understanding the development and spread of resistance between humans and animals. The high rate of ESBL-producing *E. coli* is possibly due to overuse or consistent usage of antibiotics during poultry production. Based on the results of the research and the facts presented about antimicrobial resistance as a public health problem, we point out the basic factors and strategies that would help in solving this global problem. It is very important to pay attention to people's awareness of antimicrobial resistance as well as to understand its effects on human health and the environment, then to continuously monitor and optimize the use of antimicrobials in human and animal medicine. In addition, anti-microbial resistance control measures include legal frameworks that will limit the inappropriate use of antimicrobials by farmers, as well as in human and animal medicine. It should be considered that all components of the ecosystem are interconnected. Food safety and the fight against antibiotic resistance are particularly relevant in One health approach. Only such a synergistic approach enables the complete collection of data for the rapid and efficient operation and establishment of a system for monitoring and combating antimicrobial resistance for the purpose of improving public health.

FUNDING

No specific funding was received for this study.

TRANSPARENCY DECLARATION

Competing interests: None to declare.

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