Bulgarian Journal of Veterinary Medicine, 2021, **24**, No 1, 43–56
ISSN 1311-1477; DOI: 10.15547/bjvm.2019-0084

Original article

MOLECULAR CHARACTERISATION OF EXTENDED-SPECTRUM β-LACTAMASE-PRODUCING *ESCHERICHIA COLI* AND *SALMONELLA* ISOLATED FROM POULTRY AND POULTRY PRODUCTS IN EGYPT

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Summary

Kamel, N. M., E. M. Farghaly, H. M. Shawky & A. Samir, 2021. Molecular characterisation of extended-spectrum β-lactamase-producing *Escherichia coli* and *Salmonella* isolated from poultry and poultry products in Egypt. *Bulg. J. Vet. Med.,* **24**, No 1, 43–56.

Extended-spectrum β-lactamase (ESBL) producing *E. coli* and salmonellae have spread rapidly worldwide and pose a serious threat to human and animal health. The present study was conducted to determine the prevalence of ESBL-producing *E.coli* and salmonellae, to perform molecular characterisation of the ESBL-related *bla* genes, including bla_{TEM}, bla_{SHV} and bla_{CTX}, and the susceptibilities of these bacteria to various antimicrobial agents. From a total of 300 poultry samples, 25 and 20 samples were recognised as *Salmonella* and *E. coli*, respectively by microbiological and molecular methods. All *E. coli* and *Salmonella* isolates were positive for an ESBL phenotype. Molecular detection for antibiotic resistance gene revealed bla_{TEM} in all isolates of salmonellae and *E. coli* (100%), while bla_{SHV} was detected in 5 (20%) and 2 (10%) of salmonellae and *E. coli* isolates, respectively. None of the isolates contained bla_{CTX} gene. Serotyping of *Salmonella* spp. in chickens revealed that *S. enteritidis* was the major isolates followed by *S.* Infantis (21.4%), *S.* Kentucky (14.2%) and *S.* Typhimurium, *S.* Kapemba, *S.* Newport, *S.* Vejle and *S.* Magherafelt were detected at 7.1% respectively. *S.* Infantis was the major isolate detected in chicks (60%), while in ducks *S.* Typhimurium and *S.* Blegdam were identified. In ducklings, *S.* Sinchew, *S.* Infantis and *S.* Sekondi were equally prevalent. Only *S.* Newmexico was identified in poultry products. *E. coli* in chicken were serotyped into O1, O8, O29, O125, O128 and O157. In chicks, O29 and O126 serotypes were detected. In poultry products only O8 was detected. The results indicate that ESBL frequency has reached an alarming level in poultry isolates in Egypt, with TEM enzymes being the predominant β-lactamases detected.

Key words: *E. coli*, ESBL, poultry, poultry products, *Salmonella*

INTRODUCTION

Poultry industry is one of the most widespread food industries worldwide. Chicken is the most commonly farmed species, with over 90 billion tons of chicken meat produced per year (FAO, 2017). However, there is a potential threat

of bacterial infection to poultry that can result in a huge economic loss. A lot of antimicrobials are used to raise poultry in most countries (Landers *et al.,* 2012). The same antimicrobials are considered to be essential in human medicine (WHO, 2010). The misuse of such essential antimicrobials in animal production is likely to accelerate the development of antibiotic resistance in pathogens, as well as in commensal organisms.

E. coli is commonly found in human and animal intestinal tracts and, as a result of faecal contamination or contamination during food animal slaughter, is often found in soil, water, and foods. A number of *E. coli* strains are recognised as important pathogens of colibacillosis in poultry and some of them can cause severe human diseases such as haemorrhagic colitis and haemolytic uremic syndrome (Ferens & Hovde, 2011). This reservoir of *E. coli* has a zoonotic potential that could be transferred directly from poultry to humans (Ewers *et al.,* 2009).

Salmonella infections are a major public health problem with a significant social and economic impact. Many animal species are potential reservoirs for this bacterium, especially chickens, pigeons and reptiles (Sanchez *et al.,* 2002). *Salmonella* spp. is widespread in poultry production. Prevalence varies depending on country and type of production as well as the detection methods applied. It is known to be the etiological agent of salmonellosis in both humans and animals. Food-borne salmonellosis still occurs throughout the world (Bell & Kyriakides, 2007).

The use of antibiotics in animals (Mahbub *et al.,* 2011) and poultry feed (Diarra *et al.*, 2007) disrupts normal flora of intestine, resulting in emergence of antibiotic-resistant salmonellae and *E. coli* which then find their way into the environment and food chain. Increasing resistance to antimicrobial agents remains a major challenge to public health professionals in both developed and developing countries. However, antimicrobial resistance is exacerbated by over-prescription of antibiotics and increased use in both human and animal healthcare (Da Costa *et al.,* 2013). Drug resistance is growing and has affected critically important classes of antibiotics, such as the β-lactams, which are among the most significant bactericidal antibiotics used to treat bacterial infections in humans (Livermore *et al.,* 2007).

Extended spectrum beta-lactamases (ESBLs) are enzymes produced by certain bacteria that are able to hydrolyse extended spectrum cephalosporins. They are effective against beta-lactam antibiotics such as ceftazidime, ceftriaxone, cefotaxime and oxyiminomonobactam. Globally, ESBLs are considered to be problematic and there is an increasing frequency of ESBL in different parts of the world (Ghafourian *et al.,* 2014). In veterinary medicine, a variety of these drugs is currently authorised for use, resulting in the emergence of ESBL-producing Gram-negative bacteria (Bush *et al.,* 1995). ESBL *Enterobacteriaceae* are widespread in poultry farming, but were also detected in other farm animals and their meat products (Doi *et al.,* 2010).

TEM, SHV and CTX-M are the most prevalent ESBL types (Cantón & Coque, 2006). The presence of ESBL genes has been clearly reported in food-production animals and the food chain has been described as a possible pathway from animals to humans (Leverstein-van Hall *et al.,* 2011). ESBL producing bacteria have been recovered from livestock (swine, cattle, poultry, and turkey), from companion animals (cats, dogs, horses), and from wild animals. The gastrointestinal

tract of animals is a reservoir for bacteria carrying β-lactamases and a potential source of human pathogens. In particular, ESBL has been described in healthy poultry, in faecal samples of broilers and in broiler chicken caecal samples and turkey flocks (Bortoloaia *et al*., 2011).

The occurrence of antibiotic-resistant bacteria and the presence of such bacteria on poultry farms in any country is of global significance because of the possibility of their local or even worldwide spreading by humans, animals, insect vectors, agricultural products, food products or surface water (Davies & Davies, 2010). Therefore, it is very important to monitor the resistance to antibiotics not only in human bacterial pathogens but also in pathogenic and commensal bacteria of animal origin. The aim of present study was to determine the prevalence of ESBL-producing *E. coli* and *Salmonella* and molecular characterisation of the ESBL-related *bla* genes, including bla_{TEM}, bla_{SHV} and bla_{CTX} in poultry and poultry products.

MATERIALS AND METHODS

Samples

A total number of 300 samples – 250 from different poultry organs (liver, lung, heart, intestine, yolk sac and meconium of chickens (n=120), ducks (n=40), chicks (n=30), turkeys (n=30) turkey poults (n=20), ducklings (n=10) as well as 50 samples from poultry products (fillets, nuggets, shawarma, chicken luncheon and chicken stoke cubes) were collected and tested for the presence of *E. coli* and salmonellae. The examined samples were collected under complete aseptic conditions using sterile scissor and forceps, with sterile sampling materials (bags, spatula) wearing disposable gloves and subjected to bacteriological examinations.

Isolation of Salmonella species

Samples were examined for the presence of *Salmonella* spp. according to ISO 6579:2002-COR 2004. Briefly 0.1 mL of pre-enriched culture was transferred to 9.9 mL Rappaport Vassilidis (RV) broth (CM0669, Oxoid) and incubated at 41°C for 18 to 24 h, loopful from the selective enrichment broth being inoculated onto Xylose Lysine Deoxycholate (XLD) (CM0469, Oxoid) agar and incubated at 37 °C for 18 to 24 h. The suspected isolates were stored on nutrient agar (CM0309, Oxoid) slant and kept at 4 °C for further identification by Gram's staining and other biochemical tests.

Isolation of Escherichia coli

A 0.1 mL aliquot from the pre-incubated samples in BPW was streaked onto MacConkey agar (CM0007, Oxoid) plates and incubated at 37 °C for 24 h according to Kreig *et al.* (1984). Typical *E. coli* colonies were subcultured in nutrient agar slant and incubated for 24 h at 37 °C then kept at 4 °C for further study.

Serogrouping

E. coli and salmonellae isolates were serotyped in the Reference Laboratory for Veterinary Quality Control on Poultry Production using commercially available kits (Test Sera Enteroclon, Anti-Coli, SIFIN Berlin, Germany, ISO 6579:2002).

Identification of ESBL producers

ESBL-producing isolates were screened by a phenotypic confirmatory test using cefotaxime, ceftazidime, cefotaxime/clavulanate (2:1) and ceftazidime/clavulanate (2:1) according to Clinical and Laboratory Standard Institute (CLSI, 2007). All

ESBL-producing isolates were collected for further investigation.

Antimicrobial susceptibility test

Antibiotic sensitivity was performed using Mueller Hinton Agar plates (HIMEDIA) using antibiotic discs of 15 commonly used chemotherapeutic agents. Interpretation of the results based on the diameter of the inhibition zones produced was done according to CLSI (2017). Isolates shown to be resistant to at least three different classes of agent were classified as multidrug resistant (MDR) (Kiratisin *et al.,* 2008).

Molecular identification

Extraction of DNA was done according to instructions for use of QIAamp DNA mini kit (Catalogue no.513). Briefly, 200 µL of the sample was added to 20 µL of proteinase K and 200 µL of AL lysis buffer and incubated at 56 ^{o}C for 10 min in a BiometraTsc thermal block. After incubation, 200 µL of 100% ethanol was added to the lysate and vortexed. The sample was then washed twice and centrifuged according to the manufacturer's instructions. DNA was eluted with 100 µL of elution buffer supplied in the kit.

Oligonucleotide primers used in PCR were supplied from Metabion (Germany) and Biobasic (Canada) (Table 1). The 25-µL master mix contained 12.5 µL of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µL of 20 pmol of each primer, 4.5 µL of water, and 6 µL of template DNA. The reactions were performed in Applied Biosystem 2720 thermal cyclers.

Fifteen microliters of each PCR product were loaded in each gel lane of 1.5% agarose gel (Applichem). Electrophoresis was done in 1× TBE (Tris Boric acid EDTA) buffer using 5 V/cm gradients. A 100 bp DNA ladder (Fermentas) was used to determine the fragment sizes. The PCR photos were photographed and analysed by using a gel documentation system (Alpha Innotech, Biometra, Germany) through its computer software.

RESULTS

Poultry and poultry products samples were examined for the presence of *Salmonella* and *E. coli*. Out of the total 300 samples, 25 *Salmonella* strains and 20 *E. coli* strains were detected. Three poultry products of all analysed poultry products resulted as two positive for *E. coli* and one positive for *Salmonella*.

The results of *Salmonella* serotyping are listed in Table 2. Twenty-five *Salmonella* isolates was serotyped into different serotypes. In chickens 14 serotypes were identified and *S.* Enteritidis was the most

Table 1. Oligonucleotide primers sequences sources

Microbial agent	Gene	Primer sequence (5'–3')	Length of amplified product	Reference
E. coli, Salmonella	bla_{TEM}	F:ATCAGCAATAAACCAGC R:CCCCGAAGAACGTTTTC	516 bp	Colom *et al*., 2003
	bla_{SHV}	F:AGGATTGACTGCCTTTTTG R:ATTTGCTGATTTCGCTCG	392 bp	
	bla_{CTX}	F:ATGTGCAGYACCAGTAARGTKATGGC R:TGGGTRAARTARGTSACCAGAAYCAGCGG	593 bp	Archambault *et al*., 2006

Table 2. Serogrouping of the isolated salmonellae

Examined sample	Sero grouping	Number	Percentage
Chickens (n=14)	*S.* Enteritidis	4	28.6%
	S. Infantis	3	21.4%
	S. Kentucky	2	14.2%
	S. Typhimurium	1	7.1%
	S. Kapemba	1	7.1%
	S. Newport	1	7.1%
	S. Vejle	1	7.1%
	S. Magherafelt	1	7.1%
Chicks (n=5)	*S.* Infantis	3	60%
	S. Papuana	1	20%
	S. Rosenberg	1	20%
Ducks (n=2)	*S.* Typhimurium	1	50%
	S. Blegdam	1	50%
Ducklings (n=3)	*S.* Sinchew	1	33.3%
	S. Infantis	1	33.3%
	S. Sekondi	1	33.3%
Poultry products: fillet (n=1)	*S.* Newmexico	1	100%

Table 3. Serogrouping of the isolated *E. coli*

Examined sample	Sero grouping	Number	Percentage
Chickens (n=15)	O1	2	13.3%
	O8	3	25%
	O29	1	8.3%
	O125	1	8.3%
	O128	2	13.3%
	O157	1	8.3%
	Untypable	5	41.7%
Chicks (n=3)	O29	1	33.3%
	O126	1	33.3%
	Untypable	1	33.3%
Poultry products (fillet)(n=2)	O8	1	50%
	Untypable	1	50%

predominant one while in chicks *S.* Infantis was the major isolate obtained. Another two serotypes were identified in ducks: *S.* Typhimurium and *S.* Blegdam but in ducklings three serotypes were identified: *S.* Sinchew, *S.* Infantis and *S.* Sekondi. Only *S.* Newmexico serotype was identified in poultry products (chicken fillet).

In the present study 20 *E. coli* was isolated out of total 300 examined samples (Table 3). Serotyping of the isolates by slide agglutination technique revealed the distribution of the detected isolates in different serotypes, 15 *E. coli* isolates from the chicken were serotyped into O1, O8, O29, O125, O128 and O157. In chicks two serotypes were detected: O29

Table 4. Characteristics of ESBL-producing *E.coli* isolates recovered from poultry and poultry products

Examined sample	Serotype	Number of ESBL-positive	Number of MDR-positive
Chickens (n=15)	O1 (n=2)	2	2
	O8 (n=3)	3	3
	O29 (n=1)	1	1
	O125 (n=1)	1	1
	O128 (n=2)	2	2
	O157 (n=1)	1	1
	Untypable (n=5)	5	4
Chicks (n=3)	O29 (n=1)	1	1
	O126 (n=1)	1	–
	Untypable (n=1)	1	1
Poultry products (fillet) (n=2)	O8 (n=1)	1	1
	Untypable (n=1)	1	1

Table 5. Characteristics of ESBL-producing *Salmonella* isolates recovered from poultry and poultry products.

Examined sample	Serotype	Number of ESBL-positive	Number of MDR-positive
Chickens (n=14)	*S.* Enteritidis (n=4)	4	1
	S. Infantis (n=3)	3	2
	S. Kentucky (n=2)	2	1
	S. Typhimurium (n=1)	1	1
	S. Kapemba (n=1)	1	–
	S. Newport (n=1)	1	–
	S. Vejle (n=1)	2	–
	S. Magherafelt (n=1)	1	1
Chicks (n=5)	*S.* Infantis (n=3)	3	1
	S. Papuana (n=1)	1	1
	S. Rosenberg (n=1)	1	–
Ducks (n=2)	*S.* Typhimurium (n=1)	1	–
	S. Blegdam (n=1)	1	–
Ducklings (n=3)	*S.* Sinchew (n=1)	1	1
	S. Infantis (n=1)	1	–
	S. Sekondi (n=1)	1	
Poultry products (fillet) (n=1)	*S.* Newmexico (n=1)	1	

and O126. Only O8 was detected in poultry product.

Phenotypical detection of ESBL showed that all *Salmonella* and *E.coli* isolates were positive for ESBL producing. In addition some of the ESBL-producing strains expressed an MDR phenotype (Table 4 and 5).

Molecular characterisation of ESBL genes in *Salmonella* and *E.coli* strains revealed that all were ESBL phenotype-positive. All isolates of *Salmonella* and

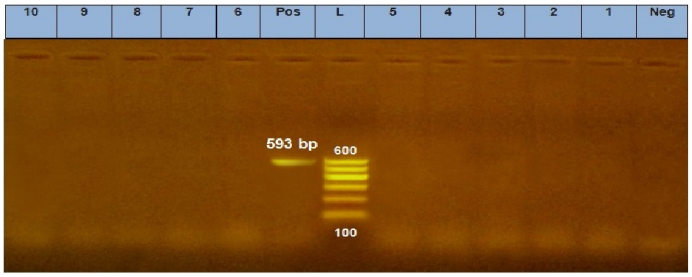

Fig. 1. Agarose gel electrophoresis of PCR for detection of bla_{CTX} gene in *Salmonella* showing amplification of 593 bp in 25 examined samples. L (Ladder): DNA ladder (100–600 bp); Lanes 1–10: negative samples; Pos: positive control; Neg: negative control.

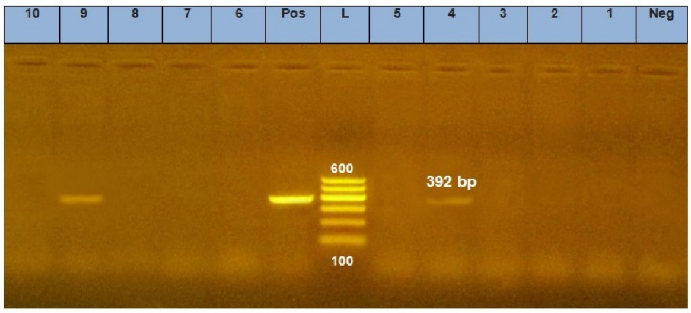

Fig. 2. Agarose gel electrophoresis of PCR for detection of bla_{SHV} gene in *E.coli* showing amplification of 392 bp in 20 examined samples. L (Ladder): DNA ladder (100–600 bp); Lanes 4, 9: positive samples. Pos: positive control; Neg: negative control.

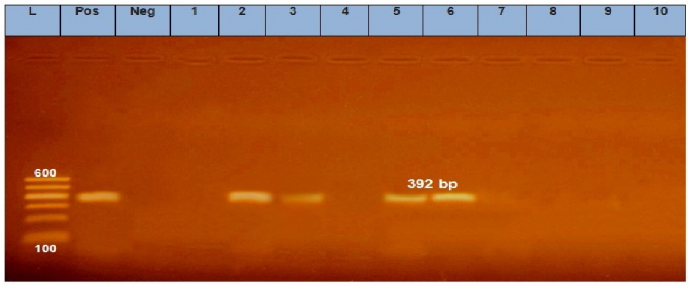

Fig. 3. Agarose gel electrophoresis of PCR for detection of *bla*SHVgene in *Salmonella* showing amplification of 516 bp in examined samples. L (Ladder): DNA ladder (100–600 bp); Lanes 2–6: positive samples. Pos: positive control; Neg: negative control.

E.coli carried the bla_{TEM} gene while bla_{SHV} was detected in 5 (20%) *Salmonella* isolates and 2 (10%) *E. coli* isolates. No bla_{CTX} was detected in salmonellae and *E. coli* (Fig 1–5).

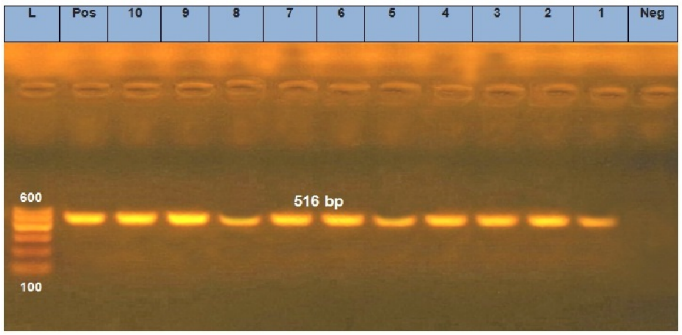

Fig. 4. Agarose gel electrophoresis of PCR for detection of *bla* $_{TEM}$ gene in *Salmonella* showing amplification of 516 bp. in examined samples. L (Ladder): DNA ladder (100–600 bp); Lanes 1–10: positive samples; Pos: positive control; Neg: negative control.

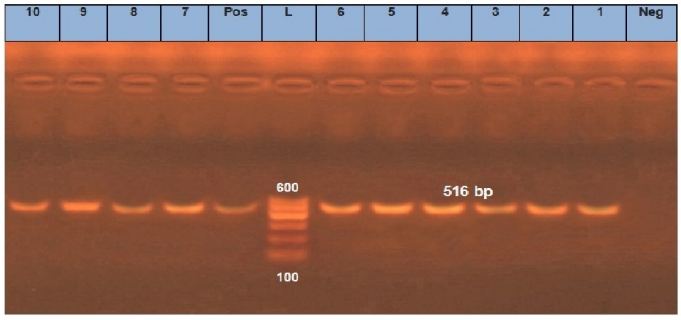

Fig. 5. Agarose gel electrophoresis of PCR for detection of *bla* $_{TEM}$ gene in *E.coli*. Showing amplification of 516 bp. in examined samples. L (Ladder): DNA ladder (100–600 bp); Lanes (1–10: positive samples; Pos: positive control; Neg: negative control.

DISCUSSION

Great attention has been paid to bacterial resistance to antibiotics in both human and animal populations for its adverse impacts on morbidity and mortality from diseases caused by resistant bacteria, economic costs of therapy and real risks of the spread of resistant strains among animals and humans (White, 2006). With the extensive use in poultry of β-lactam antibiotics such as amoxicillin and cephalosporins, especially extended-spectrum cephalosporins, ESBL-mediated resistance in Gram-negative bacilli has become increasingly critical, and therapeutic options for such infections – limited (Pfaller & Segreti, 2006). It is important to detect ESBL producers in order to know the ESBL prevalence in animal-associated bacteria and to limit the spread of these MDR organisms in veterinary settings (Liu *et al.,* 2007).

Salmonella infections are a major public health problem with a significant social and economic impact. Many animal species are potential reservoirs for this bacterium, especially chickens, pigeons and reptiles (Sanchez *et al.,* 2002). Chickens and chicken products played an important

role in *Salmonella* contamination and are considered the main cause of salmonellosis that causes enteritis in human beings. Moreover, the most important source for *Salmonella* infection in humans is handling poultry carcasses as well as consumption of undercooked poultry meat (Panisello *et al.,* 2000).

In the present study *Salmonella* isolates are classified into different serotypes with variation in the percentage. This variation in result may be attributed to different breeds of chickens, different ages, and collection of samples during different seasons. Twenty five distinct *Salmonella* serotypes were identified; *S.* Enteritidis was the most frequent isolates in chicken, while in chicks *S.* Infantis was the major isolates. Also two other serotypes were identified in ducks: *S.* Typhimurium and *S.* Blegdam. In ducklings, three different serotypes were identified: *S.* Sinchew, *S.* Infantis, *S.* Sekondi and only *S.* Newmexico was detected in poultry product. Our data are similar to those of Ammar *et al.* (2010) who isolated *S.* Enteritidis from broiler chicken and chicken meat from Egypt. Hugo & Russel (1998) reported that *Salmonella* spp. were isolated from poultry in Brazil with a prevalence of 2.7% and the most common isolates were *S.* Enteritidis (48.8%), *S.* Infantis (7.6%), *S.* Typhimurium (7.2%), and *S.* Heidelberg (6.4%). Gwida & El-Gohary (2015) isolated *S.* Kentucky at high rate followed by *S.* Enteritidis*, S.* Infantis and *S.* Typhimurium. The association of specific serotypes with poultry might be related to host adaptation or other unknown factors (Uzzau *et al.,* 2000).

E. coli is one of the most common causes of infection by Gram negative bacteria (Diekema *et al.,* 1999). Avian pathogenic *E. coli* is responsible for causing colisepticaemia, coligranuloma and air sacculitis (Sams, 2001). In the current study serotyping of the obtained *E. coli* isolates revealed that O8 serotype was the predominant one. Our finding disagree with that of Eid *et al*. (2015) who reported that O27 was the predominant *E. coli* serotypes at a rate of 22% among the 13 identified serotypes. From the other hand serotyping of *E.coli* with the available kits failed to identify 7 isolates in this study. This finding agreed with that of Rosario *et al*. (2004) who failed to identify the serogroups of 15% of isolates. Also Eid *et al*. (2015) failed to identify 5 (14%) of the *E. coli* isolates. There were variations in the distribution of serotypes according to geographic regions. Many serotypes have been found less frequently, while some APEC do not belong to known serotypes or are untypeable (Swayne, 2013).

The high prevalence of ESBL-producing *E. coli* and *Salmonella* (100%) in poultry and poultry products in this study was in accordance with another survey on *E. coli* ESBL producers in Egypt (Ghafourian *et al.,* 2012) indicating that 38% of the *E. coli* tested positive for ESBLs. Similarly, in Netherlands Kola *et al.* (2012) found that 100% of conventional and 84% of organic samples were ESBL producers. In this study, Ziech *et al.* (2016) demonstrated multidrug resistance in 86% and ESBL activity in 45% of the *Salmonella* spp. isolated from the studied broiler processing plants. In this regard, ESBL contamination might occur during the rearing process, slaughtering or finally, through the environment (Stuart *et al.,* 2012).

In fact, Gregova (2012) showed that the occurrence of ESBLs in poultry meat could be related to the environmental microbes of the slaughterhouse, to scalding, defeathering, and evisceration processes, and to transfer of bacteria from chickens

because of the contact through water and incorrect cleaning and disinfection. In addition, some of the ESBL-producing strains expressed an MDR phenotype, suggesting that ESBL-producing isolates have probably acquired additional resistance genes. In order to gain a better understanding of their genetic relationships, further studies are needed to characterise the plasmids on which *bla* genes and other resistance genes are located.

Resistance to β-lactam antimicrobial agents in Gram-negative bacilli is primarily mediated by β-lactamases. Although a variety of β-lactamases has been described, the TEM, SHV and CTX enzymes are those most frequently observed among members of the family *Enterobacteriaceae* (Livermore, 1995). Extended-spectrum β-lactamases (ESBLs) are considered one of the most important resistance mechanisms for penicillins and cephalosporins when these enzymes are produced in *E. coli*, *Salmonella* spp. and *Klebsiella* spp. (Bush, 2001).

A high incidence of bla_{TEM} detection in *E. coli* and *Salmonella* (100%) in the present study was previously recorded. Colom *et al*. (2003) detected bla_{TEM} gene in 45 out of 51 (88.2%) amoxicillin-clavulanate resistant *E. coli* isolates. Brinas *et al.* (2002) detected the bla_{TEM} gene in 83% of 124 ampicillin resistant *E. coli* isolates including food isolates of chicken origin. Adelowo *et al.* (2014) found resistant genes in *E. coli* isolates from Nigeria and these included bla_{TEM} (85%). Ghazaei (2018) isolated 20 *Salmonella* strains from 100 samples of commercial broilers and spent hens among which bla_{TEM} was found to be the dominant β-lactamase gene (85%), followed by $bla_{CTX\text{-}M}$ (60%) and bla_{SHV} (35%); 100% of the isolates were found to be ESBL producers. The low incidence of bla_{SHV} in the present study is consistent with the report of Dandachi *et al.* (2018) who analysed 981 faecal swabs from 49 poultry farms in Lebanon. Out of 235 strains isolated, 217 were identified as *E. coli* (92%). The putative TEM gene was detected in 83% of the isolates, SHV in 20%, and CTX-M in 53%. Yuan *et al.* (2009) performed molecular characterisation of ESBL and showed that none of the isolates contained the bla_{SHV} gene. Our results disagree with those of Kar *et al.* (2015) who detected beta-lactamase genes in *E.coli* viz., bla_{SHV} , $bla_{CTX\text{-}M}$, bla_{TEM} and *blaampC* were detected in 17, 13, 9 and 2 isolates, respectively through PCR. The SHV was more prevalent than the other types of ESBLs in clinical isolates of bacteria (Jacoby, 1997).

None of the *Salmonella* and *E. coli* isolates carried the bla_{CTX} gene. These results disagree with Cantón & Coque (2006) who mentioned that the main ESBL types were TEM, SHV, and CTX-M. Rates of CTX-M infections have increased during the last decade compared with rates of TEM and SHV infections. Djeffal *et al.* (2017) reported that 18 of *Salmonella* strains (12 avian and 6 human) were found to produce ESBLs, identified as members of the blaCTX-M-1 (n=12), blaCTX-M-15 (n=5) and blaTEM group (n=8). Two reasons for the absence of these genes might be the relatively small sample size and the exclusive origin of the poultry.

CONCLUSION

The findings from this work support the need for a critical review of the usage of antimicrobial agents in livestock in Egypt and the importance of taking specific steps to curtail the indiscriminate use of antimicrobial agents to prevent the possi-

ble adverse consequences in animal production, as well as in humans. There is an urgent need to formulate a strategy and put the necessary plan in place to execute a policy targeted at the promotion of rational use of antimicrobial agents, as an important element in antimicrobial resistant containment.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Heba Bader, Ahmed Sayed, Hend Karm and the staff of microbiology unit at Reference Laboratory of Poultry Production (RLQP) for their kind support.

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Paper received 05.06.2019; accepted for publication 13.08.2019

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