Original Article

Veterinary Research Forum. 2023; 14 (3) 131 - 138

doi: 10.30466/vrf.2021.539418.3233

Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Prevalence, virulence factors, and antimicrobial resistance profiles of Shiga toxin-producing *Escherichia coli* isolated from broiler chickens in Egypt

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Article Info

Article history:

Received: 21 September 2021
Accepted: 20 December 2021
Available online: 15 March 2023

Keywords:

Antimicrobial resistance
Chickens
STEC
Virulence

Abstract

Pathogenic *Escherichia coli* is one of the world's most important zoonotic foodborne pathogens and poses a serious threat to public health. We examined the prevalence, virulence genes, and antibiotic resistance profile of Shiga toxin (Stx)-producing *E. coli* (STEC) isolated from broiler chickens in the Kafr El-Sheikh governorate, Egypt. A total of 410 samples (230 cloacal swabs, 180 internal organs) were collected to isolate *E. coli*. A total of 29 (7.07%) *E. coli* isolates were recovered and identified, and 18 of them harbored *Stx* genes (*stx*). Out of 18 isolates, five (17.24%) carried the *stx1* gene, five (17.24%) carried the *stx2* gene, four (13.79%) carried both *stx1* and *stx2* genes, and four (13.79%) carried *stx1, stx2,* and *eaeA* genes. Overall, complete antibiotic resistance was observed against amoxicillin, ampicillin, cefpodoxime, and cefoperazone; high resistance was observed against ampicillin/sulbactam, nalidixic acid, cefuroxime, aztreonam, ciprofloxacin, ceftriaxone, chloramphenicol, sulfamethoxazole/trimethoprim, and ceftazidime; moderate resistance against gentamicin; low resistance against cefoxitin; lower resistance was detected against norfloxacin, cefotetan, and amikacin; and the lowest resistance against imipenem. All *E. coli* isolates demonstrated multidrug resistance against at least four antibiotic classes. Out of 29 *E. coli* isolates, STEC accounted for 18 isolates, of which the O78, O26:H11, O128:H2, O1:H7, O119:H6, and O91:H21 serogroups were predominant. All *E. coli* isolates were multidrug resistant and therefore pose a potential public health concern as these virulent, resistant strains may spread to humans. Thus, high levels of hygiene and biosecurity are required by chicken handlers to decrease the danger of infection spreading to humans.

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Introduction

Infection by *Escherichia coli* is a serious problem that can cause a high economic loss for poultry and bird-related industry enterprises worldwide.[1] Several different *E. coli* infections have been documented including yolk sac infection, omphalitis, cellulitis, swollen head syndrome, coligranuloma, and colibacillosis.[2] Infected poultry is one of the most important sources of disease transmission, and infectious diseases caused by pathogens are the main cause of death of chickens and humans.[3] One source of foodborne diseases in humans is the consumption of infected broilers.[4] The four major foodborne diarrheagenic *E. coli* pathotypes are enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), and Shiga toxin (Stx)-producing *E. coli*/enterohemorrhagic *E. coli*/verocytotoxin-producing *E. coli* (STEC /EHEC/VTEC), which are categorized depending on their virulence determinants, host cell attachment patterns, and consequences, as well as toxin synthesis and invasiveness of the bacteria;[5] *E. coli* that causes acute infectious diarrhea in children in developing countries has been recognized as diarrheagenic *E. coli* (DEC).[6]

The STEC isolates are recognized for their capacity to generate Stxs.[7] These toxins are key virulence elements that cause bloody diarrheal disease and can develop into life-threatening systemic complications such as an acute renal failure syndrome, referred to as a hemolytic uremic syndrome (HUS), and central nervous system abnormalities.[8] The STEC are an EHEC subgroup that are most commonly associated with foodborne disease outbreaks and cause hemorrhagic colitis.[9] The Stx family includes two major immunologically non-cross-reactive groups of toxins, *Stx*1 and *Stx*2, whose genes (*stx1* and *stx2*,

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respectively) are encoded on a lysogenic lambdoid bacteriophage. A single EHEC strain can express one or both toxins, although *stx2* is more effective in inducing cytotoxicity and more dangerous in the development of HUS than *stx1*.[10] The pathogenesis of STEC is not primarily caused by the *Stx* genes; a number of other virulence factors encoded by genes located on mobile genetic elements also play a substantial role.[11] A critical virulence factor is the outer membrane protein called intimin, encoded by *eaeA*, which is believed to determine the formation of attaching and effacing lesions.[12]

The *E. coli* O157:H7 strains have been identified as the source of outbreaks and sporadic symptomatic and asymptomatic illnesses globally.[9] However, other non-O157 serotypes have also been related to serious human diseases.[13] Currently, there is a global emergence of non-O157 STEC, which are more prevalent causes of acute diarrhea than the more well-known O157 strains and can create massive outbreaks.[9] The Centers for Disease Control and Prevention predicted that 265,000 STEC diseases occur annually in the United States, with non-O157 STEC strains accounting for 64.00% of these infections indicating that non-O157 STEC strains are a serious public health risk.[14] Furthermore, non-O157 STEC are known to be responsible for 81.00% of all STEC gastroenteritis and 32.00% of STEC-related HUS cases in Germany between 2008 and 2012.[15] Non-O157 STEC may account for 20.00% to 70.00% of all STEC global infections.[16]

Continued use of antibiotics in farms is believed to be a low-cost prevention strategy but it increases the factors contributing to the emergence of antibiotic resistance in poultry and consequently human food chain.[4] Additionally, increasing pathogenicity, virulence,[17] and continuing virulence evolution may all be contributing factors to the threat posed by antibiotic-resistant bacteria.[18] Thus, in addition to preventing antibiotic resistance, we must also prevent virulence from spreading.[19] However, the rate of discovery of new antibiotics is slower than the rate of increased antibiotic resistance raising concerns that there would eventually be no effective antibiotics available to treat resistant bacterial infections.[20] *E. coli* isolates were classified according to their antimicrobial resistance profiles into pan drug-resistant (PDR) when they showed resistance to all antibiotics in all antibiotic classes examined, extensively drug-resistant (XDR) when they are resistant to all antibiotic classes tested except one or two antibiotic classes, and multidrug-resistant (MDR) if they exhibited resistance to at least one antibiotic agent in three or more antibiotic classes.[21] Multidrug resistance (MDR) occurs when an isolate gains resistance to three or more antibiotic groups.[21] The development of MDR in *E. coli* strains has provoked global alarm. MDR bacteria have been discovered in individual chickens and are believed to have originated from farms that continue to overuse antibiotics.[22] To date, scant research has been conducted on STEC in broiler chickens; therefore, the primary objectives of this study were to determine the prevalence of non-O157 *E. coli* in broiler chickens in the Kafr El-Sheikh governorate, Egypt, and their antimicrobial resistance profile, and to assess the virulence genes of the isolates using conventional PCR assays.

Materials and Methods

Sample collection. A total of 410 samples were collected between March and July 2021 from diseased broiler chickens (1 to 42-days-old) from different localities in the Kafr El-Sheikh governorate, northern Egypt. Collected samples included 230 cloacal swabs and 180 internal organ samples (liver, spleen, kidney, gall bladder, yolk sac, and fresh heart blood). The samples were labeled and transferred immediately in an icebox to the bacteriology laboratory at the Faculty of Veterinary Medicine, Kafrelsheikh University.

Isolation and identification procedures. A loopful from MacConkey broth (HiMedia, Mumbai, India) of each sample was streaked onto a Sorbitol MacConkey agar (HiMedia) and Cefixime Tellurite Sorbitol MacConkey agar (HiMedia) and was then incubated at 37.00 ˚C for 18 - 24 hr. Bacterial colonies exhibiting *E. coli* characteristics were stained and confirmed as *E. coli* by using a panel of biochemical tests developed by MacFaddin,[23] which included gas production, sugar fermentation, motility, urease production, lysine decarboxylation, IMViC tests (indole, methyl red, Voges-Proskauer, and citrate utilization tests), hydrogen sulfide production, gelatin hydrolysis, nitrate reduction, and detection of ornithine decarboxylase. The confirmed *E. coli* isolates were stored in nutrient broth with 30.00% sterile glycerol at – 80.00 ˚C.

Serological identification of *E. coli*. Confirmed *E. coli* isolates were serologically identified according to Kok *et al*.[24] by using the rapid diagnostic *E. coli* antisera sets (Denka Seiken Co., Ltd., Tokyo, Japan) for diagnosis of the pathogenic types using polyvalent and monovalent diagnostic *E. coli* antisera.

Molecular detection of virulence genes of *E. coli*. All confirmed *E. coli* isolates were subcultured on Luria-Bertani broth medium (Merck, Darmstadt, Germany). After 18 - 20 hr of incubation at 37.00 ˚C, genomic DNA was extracted by boiling as described previously.[25] All strains were examined via multiplex PCR for the *stx1*, *stx2*, and *eaeA* genes according to Paton and Paton.[26] Table 1 summarizes the primer sequences, target genes, amplicon sizes, and their relevant references. The *E. coli* reference strains were O157:H7 Sakai (positive for *stx1*, *stx2*, and *eaeA*) and K12 DH5α (a nonpathogenic negative control strain with no virulence genes). Amplification was performed on a thermal cycler (Master Cycler; Eppendorf, Hamburg, Germany). Exactly 20.00 ng of chromosomal DNA was used for amplification in 25.00 µL of buffer solution containing

Table 1. Primer sets for PCR amplification of the virulence genes in *E. coli* isolates.[26]

Genes	Primer, oligonucleotide sequence (5′ → 3′)	Product size (bp)
Shiga toxin1 (*stx1*)	F: 5′ATAAATCGCCATTCGTTGACTAC′3 R: 5′AGAACGCCCACTGAGATCATC′3	180
Shiga toxin 2 (*stx2*)	F:5′ GGCACTGTCTGAAACTGCTCC′3 R: 5′TCGCCAGTTATCTGACATTCTG′3	255
Intimin (*eaeA*)	F: 5′ GACCCGGCACAAGCATAAGC ′3 R: 5′ CCACCTGCAGCAACAAGAGG ′3	384

3.00 µM oligonucleotides, 200 µM of each deoxynucleoside triphosphate, 3.50 mM $MgCl_2$, and 2.50 U DNA Taq polymerase (Life Technologies, Rockville, USA). Samples were cycled 35 times with 60 sec denaturation at 95.00 ˚C; 120 sec annealing at 65.00 ˚C during the first 10 cycles, decreasing to 60.00 ˚C by cycle 15; and 90 sec elongationS at 72.00 ˚C, increasing to 150 sec from cycles 25 to 35. The PCR-amplified products were electrophoresed in 2.00% (w/v) agarose gels and stained with 0.50 mg of ethidium bromide per milliliter for 15 min. The gel was then visualized and photographed under a UV transilluminator.

Antimicrobial susceptibility test. The isolates were tested *in vitro* for their susceptibility to 19 antimicrobial agents (Oxoid, Hampshire, UK) using a Kirby–Bauer disc diffusion assay according to the standards and interpretive criteria described by Clinical Laboratory Standard Institute guidelines.[27] The following groups of anti-microbial agents were tested: penicillin group, including ampicillin (AMP), 10.00 µg, and amoxicillin (AMX), 25.00 µg; cephalosporin group, including cefuroxime (CXM), 30.00 µg; cefotetan (CTT), 30.00 µg; cefoxitin (FOX), 30.00 µg; cefpodoxime (CPD), 10.00 µg; cefoperazone (CPZ), 72.00 µg; ceftriaxone (CRO), 30.00 µg; and ceftazidime (CAZ), 30.00 µg; carba-penem group, including imipenem (IMP), 10.00 µg; mono-bactam group, including aztreonam (ATM), 30.00 µg; β-lactamase inhibitor group, including ampicillin/sulbactam (SAM), 10.00/10.00 µg; aminoglycoside group, including gentamicin (GEN), 10.00 µg, and amikacin (AMK), 30.00 µg; phenicols group, including chloramphenicol (CHL), 30.00 µg; fluoroquinolone group, including ciprofloxacin (CIP), 5.00 µg, and norfloxacin (NOR), 10.00 µg; quinolone group, including nalidixic acid (NAL), 30.00 µg; and folate path-way antagonist group, including sulfamethoxazole/tri-methoprim (SXT), 23.75/1.25 µg. Results were recorded as sensitive or strain ATCC 25922 was used as a quality control.

Results

Prevalence of *E. coli* among examined samples. A total of 29 (7.07%) *E. coli* isolates were recovered and identified out of 410 samples analyzed from diseased broiler chickens. *E. coli* isolates were observed in 8.70% (20/230) of cloacal swabs and 5.00% (9/180) of internal organs (liver, spleen, kidney, and yolk sac, two isolates each) and one isolate from heart blood. However, no isolates were recovered from the gall bladder. Table 2 shows the distribution of *E. coli* isolates and their serotypes among various sample sources.

Serotyping of *E. coli* isolates. Isolate serotyping identified that the STEC belonged to 12 different serogroups (Table 2) and revealed the predominance of serotypes, which included O78 (seven isolates), O26:H11 and O128:H2 (four isolates each), O1:H7 (three isolates), O91:H21 and O119:H6 (two isolates each), and one isolate each for O146:H21, O121:H7, and O44:H18. These results revealed that the EPEC strains were the most predominant pathotype (16 isolates; 55.17%), followed by EHEC (six isolates; 20.69%), ETEC (four isolates; 13.79%), and EIEC *E. coli* (three isolates; 10.35%). The prevalence of the pathogenic strains was 8/9 (88.90%) from internal organs and 10/20 (50.00%) from cloacal swabs.

Molecular detection of virulence genes of sero-typed isolates. Multiplex PCR was used to examine all *E. coli* isolates for STEC virulence genes. The molecular screening detected *Stx* genes in 18 isolates (62.10%). Five (17.24%) of these isolates carried *stx1*, 5(17.24%) carried *stx2*, 4(13.79%) were positive for both *stx1* and *stx2* genes, and 4 (13.79%) carried *stx1*, *stx2*, and *eaeA* genes. Notably, 11(37.93%) isolates did not carry any of examined genes. Table 3 and Figure 1 show the virulence gene distribution profile for the STEC strains isolated from broiler chickens.

Table 2. Distribution of serotyped *E. coli* in various samples.

Sample type	Number of *E. coli* isolates (%)	Serotypes *E. coli* (number of isolates)
Liver	2/34 (5.88)	O26:H11 (1), O91:H21 (1)
Spleen	2/34 (5.88)	O26:H11 (1), O78 (1)
Kidney	2/34 (5.88)	O119:H6 (1), O146:H21 (1)
Heart blood	1/34 (2.94)	O128:H2 (1)
Gall bladder	0/34 (0.00)	-
Yolk sac	2/10 (20.00)	O91:H21 (1), O128:H2 (1)
Cloacal swab	20/230 (8.70)	O26:H11 (2), O119:H6 (1), O128:H2 (2), O121:H7 (1), O44:H18 (1), O1:H7 (3), O78 (6) O2:H6 (1), O124 (1), O159 (2)
Total	29/410 (7.07)	12 serogroups

Table 3. Distribution of virulence genes profile of STEC strains isolated from broiler chickens.

Virulence genes	Number of *E. coli* isolates (%)	O Serotypes *E. coli* (number of isolates)
Shiga toxin1 (*stx1*)	5/29 (17.24)	O44:H18 (1), O128:H2 (4)
Shiga toxin 2 (*stx2*)	5/29 (17.24)	O121:H7 (1), O1:H7 (3), O146:H21 (1)
stx1* and *stx2	4/29 (13.79)	O119:H6 (2), O91:H21 (2)
***stx1*, *stx2*, and intimin (*eaeA*)**	4/29 (13.79)	O26: H11 (4)

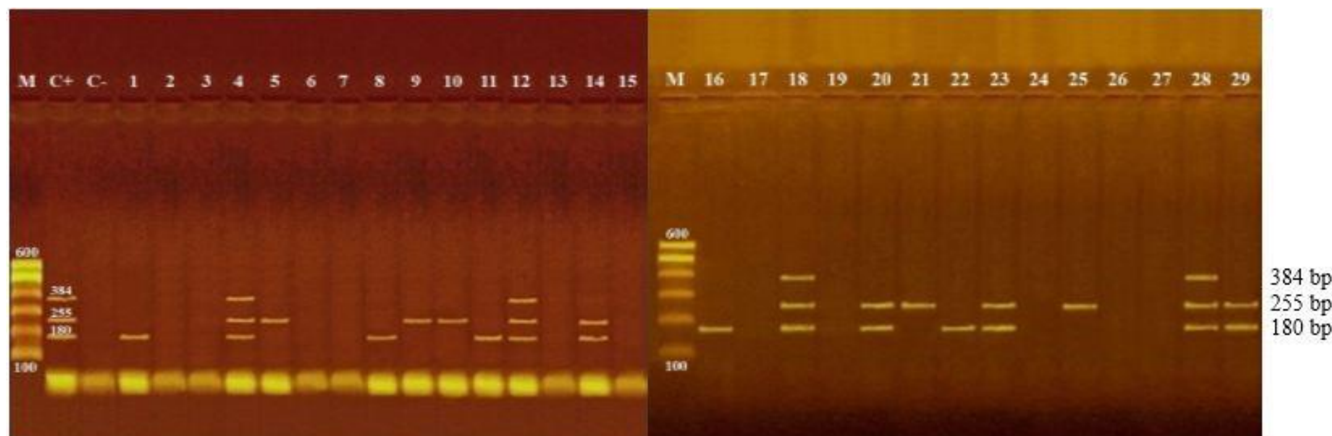

Fig. 1. Agarose gel electrophoresis of multiplex PCR of *stx1*, *stx2*, and, *eaeA* (180, 255, and 384 bp, respectively) virulence genes for characterization of enteropathogenic *E. coli*. Lane M: 100 bp ladder DNA marker. Lane C+: control positive for *stx1*, *stx2*, and *eaeA*. Lane C−: negative control for *stx1*, *stx2*, and, *eaeA*. Lanes 1, 8, 11, 16 and, 22: positive isolates for *stx1*. Lanes 5, 9, 10, 21 and, 25: positive isolates for *stx2*. Lanes 14, 20, 23, and, 29: positive isolates for *stx1*, and, *stx2*. Lanes 4, 12, 18 and, 28: positive isolates for *stx1*, *stx2*, and, *eaeA*. Conversely, lanes 2, 3, 6, 7, 13, 15, 17, 19, 24, 26 and, 27: negative isolates for *stx1*, *stx2*, and, *eaeA*.

Antimicrobial resistance profile of *E. coli* isolates. Overall, complete antibiotic resistance was observed against AMX, AMP, CPD, and CPZ; high resistance was observed against SAM, NAL, CXM, ATM, CIP, CRO CHL, SXT, and CAZ; moderate resistance against GEN; low resistance against FOX; lower resistance was detected against NOR, CTT, and AMK; and the lowest resistance against IMP (Fig. 2). The resistance of the isolates to different antibiotics was recorded. Each isolate was resistant to AMP, AMX, CPD, and CPZ. All isolates tested exhibited MDR properties. There was a range of resistance to the 10 different classes of antibiotics: one isolate (3.45%) was resistant to four classes (MDR), three (10.35%) were resistant to six classes (MDR), three (10.35%) were resistant to seven classes (MDR), eight (27.60%) were resistant to eight classes (XDR), 13 (44.83%) were resistant to nine classes (XDP), and one isolate (3.45%) was resistant to all 10 antibiotic classes (PDR).

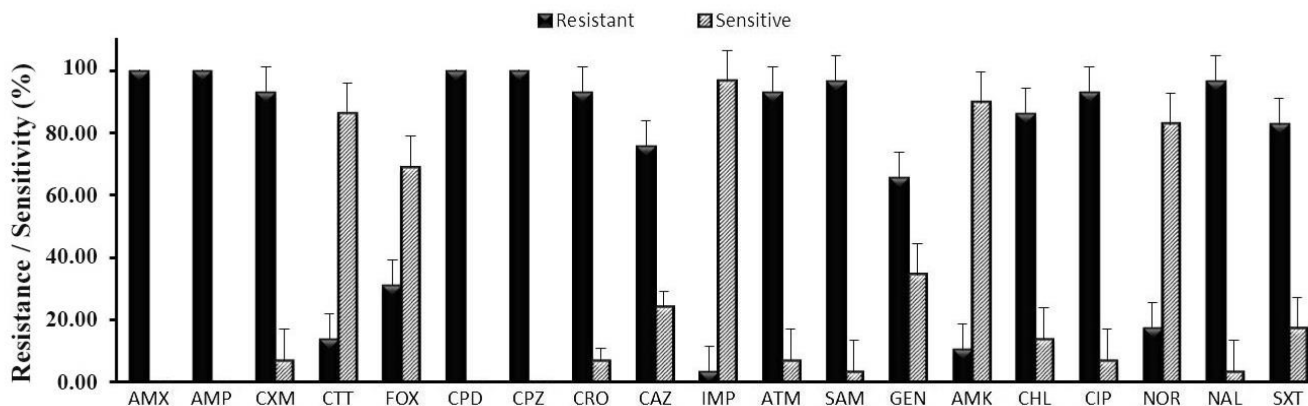

Fig. 2. Overall antibiotic resistance pattern of *E. coli* isolates in broiler chickens. AMX: amoxicillin; AMP: ampicillin; CXM: cefuroxime; CTT: cefotetan; FOX: cefoxitin; CPD: cefpodoxime; CPZ: cefoperazone; CRO: ceftriaxone; CAZ: ceftazidime; IMP: imipenem; ATM: aztreonam; SAM: ampicillin/sulbactam; GEN: gentamicin; AMK: amikacin; CHL: chloramphenicol; CIP: ciprofloxacin; NOR: norfloxacin; NAL: nalidixic acid; SXT: sulfamethoxazole/trimethoprim.

Discussion

From a zoonotic point of view, STEC is the only pathogenic *E. coli* of considerable concern because Stx-producing strains can cause serious illness in humans when transferred down the food chain from their animal reservoirs.[28] In this study, based on morphological, biochemical, and serological characteristics, the overall prevalence of *E. coli* was 7.07%. This recovery rate was lower than 78.86% achieved by Jakaria *et al*.[29] The recovery rate of *E. coli* isolates varies depending on the source of the tested samples. Our results revealed a higher incidence of *E. coli* isolates from cloacal swabs (8.70%) than from internal organs (5.00%). The incidence of recovered *E. coli* from broiler cloacal swabs (8.70%) was lower than those found in earlier studies.[22,30] The percentage of *E. coli* isolates (5.00%) from broiler internal organs was lower than previous results.[29] One fresh heart blood out of 34 samples was *E. coli* positive (2.94%) which was lower than those recorded by Younis *et al*. (23.28%).[31] A lower percentage of isolates (5.88%) was recorded for liver, spleen, and kidney samples in comparison with results reported by Kaoud *et al*. (11.00%),[30] Younis *et al*. (27.39%),[31] and El-Mongy *et al*. (10.00%),[32] respectively. Furthermore, only 20.00% of yolk sac samples were positive for *E.coli* compared with 75.20% reported by Yousef *et al*.[33] Thus, the incidence of *E. coli* in our study differs from that of other researchers which may be attributed to variations in the sampling source, type of samples, environment conditions, and hygienic and the sanitary conditions in different chicken farms.

In this study, 12 different serogroups of *E. coli* were isolated: O78 (24.14%), O26:H11 (13.79%), O128:H2 (13.79%), O1:H7 (10.35%), O91:H21 (6.89%), O119:H6 (6.89%), and O159 (6.89%) and O146:H21, O44:H18, O121:H7, O2:H6, and O124 (all at 3.35%). In contrast to our results, Kaoud *et al*.[30] reported a range of *E. coli* serotypes that had been previously isolated from broilers, including O78 (31.81%), O2:H6 (18.18%), O1:H7 (15.90%), O91:H21 (11.36%), O128:H2 (9.09%), O26:H11 (4.54%), O146:H21 (2.27%), O124 (2.27%), and O44:H18 (2.27%). In Younis *et al*.[31] the most common *E. coli* serotypes were O78 (17.80%), O1:H7 (9.50%), O2:H6 (9.50%), O91:H21 (5.40%), O26:H11 (4.00%), O44:H18 (4.00%), O124 (2.70%), O128:H2 (2.70%), O121:H7 (1.30%), O146:H21 (1.30%), O119:H6 (1.30%), and O159 (1.30%).

Shiga toxins, as virulence factors, play a key role in the pathogenicity and severity of STEC.[34] The presence of both *stx2* and *eaeA* is linked to an increased risk of severe clinical symptoms. In this study, 8/9 (88.90%) of isolates from internal organs and 10/20 (50.00%) of isolates from cloacal swabs carried at least one virulence gene, whereas in Dutta *et al*.[35] 14 strains (33.33%) harbored at least one virulence gene. Only 17.24% of our STEC isolates carried just *stx1*. These findings agreed with those of Mamun *et al*.[36] but differed from those of El-Mongy *et al*.[32] and Himi *et al*.[37] who discovered two strains that carried *stx1* only and no samples that only carried *stx1*, respectively. We found that 17.24% of our *E. coli* isolates carried *stx2*. These results are lower than those of Himi *et al*.[37] and Mamun *et al*.[36] who respectively reported 6/60 (10.00%) and 26/49 (53.06%) *stx2*-positive isolates from cloacal swabs. These percentages were higher than those found by Dutta *et al*.[35] who identified two isolates that carried *stx*2. Only 13.79% (four isolates) of our *E. coli* isolates carried *stx1* and *stx2* compared with El-Mongy *et al*.[32] who reported three isolates that carried both genes, although Mamun *et al*.[36] identified that 12.24% of their isolates carried both genes. In this study, the *stx1*, *stx2*, and *eaeA* genes were found in 13.79% of the *E. coli* tested. By contrast, Wani *et al*.[38] found that only 2.49% of *E. coli* isolates from chickens carried the *eaeA* gene, whereas Jamshidi *et al*.[39] reported that only 1.28% (one isolate out of 78) carried *eaeA* and that no isolates carried either *stx1* or *stx2*. The high percentage of the *eaeA*-carrying *E. coli* isolates in this study implies there was a significant amount of contamination.

The misuse of antibiotics in agricultural practices leads to the emergence of foodborne bacterial resistance in both humans and animals.[40] Furthermore, treatment of STEC infections with antibiotics is contentious because of the possibility of promoting the release of Stx, which might lead to HUS in humans.[41] However, antimicrobials given early in infection may help prevent the development of STEC illness to HUS.[42] Data from this study indicate that the resistances against AMX, AMP, ATM, CIP, SXT, CAZ, GEN, FOX, NOR, AMK, CXM, CRO, and IMP to *E. coli* isolates were 100%, 100%, 93.10%, 93.10%, 82.76%, 75.86%, 65.52%, 31.03%, 17.24%, 10.35%, 6.90%, 6.90%, and 3.45%, respectively. Aabed *et al*. found that resistance rates of *E. coli* to the above antibiotics were 70.80%, 70.80%, 16.70%, 41.70%, 50.00%, 16.70%, 8.30%, 12.50%, 66.70%, 0.00%, 29.20%, 20.30%, and 0.00%, respectively,[43] and Moawad *et al*. reported resistances to SXT, GEN, CAZ, CIP, AMK, and IMP as 64.30%, 19.60%, 41.10%, 21.40%, 10.70%, and 1.80%, respectively.[44] The resistances here to CPZ and NAL were 100% and 96.60%, respectively, although these results do not agree with those from Sohail *et al*.[45] who reported 72.00% and 91.00% resistances, respectively. Furthermore, we detected resistances of 96.60% and 13.80% against SAM and CTT, respectively, in contrast to Hui *et al*.[46] where the resistances to these antibiotics were 32.00% and 3.23%, respectively. Additionally, Sohail *et al*.[45] found 84.00% resistance against SAM. In the present investigation, *E. coli* isolate resistance to CPD was 100%, which is in contrast to Kwoji *et al*.[47] who found 67.70% resistance. Furthermore, our *E. coli* isolates were highly resistant to CHL (86.20%), in contrast to Amer *et al*.[22] and Effendi *et al*.[4] who found 23.00% and 65.00% resistances, respectively. The MDR in

pathogens has been identified as a major public health concern worldwide.[48] In this study, MDR in *E. coli* isolates (100%) was more widespread than those previously reported (91.80% and 69.30%) by Aworh *et al*.[49] and Mgaya *et al*.[50] respectively. The antibiogram results in this study differ from those of other research, showing that the distribution of antibiotic resistance changes with various isolates, time, and development of MDR among different *E. coli* isolates.

In this study, we isolated 29 *E. coli* samples from broiler chickens which contained 20 isolates from cloacal swabs, nine from internal organs (liver, spleen, kidney, and yolk sac, two isolates each), and one isolate from heart blood. The O78, O26:H11, O128:H2, O1:H7, O119:H6, and O91:H21 serogroups were the predominant serogroups. Using multiplex PCR, 18 *E. coli* isolates were classified as STEC (non-O157). Furthermore, the higher prevalence of STEC isolates in broiler chickens may serve as a reservoir for transmission of STEC to environment and humans. All isolated *E. coli* had substantial MDR to most of the antimicrobial agents commonly used in Egypt which was recorded as each isolate was resistant to AMP, AMX, CPD, and CPZ. The isolated *E. coli* has a range of resistance to the 10 different classes of antibiotics from complete resistance to all 10 antibiotic classes (one isolate, PDR) to the lowest range that resists four classes (one isolate, MDR), in-between three were resistant to six classes (MDR), three were resistant to seven classes (MDR), eight were resistant to eight classes (XDR), 13 were resistant to nine classes (XDR). Therefore, the uncontrolled use of antibiotics for the treatment of STEC infections in broiler chickens poses a public health concern.

Acknowledgments

We would like to sincerely thank Prof. Ashraf M. Ahmed and Prof. Ibrahim E. Eldesoukey, Department of Microbiology, Faculty of Veterinary Medicine, Kafrelsheikh University for their help and support in this research.

Conflict of interest

The authors declare no financial or conflict of interest regarding this study that could inappropriately influence the work.

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