Prevalence of Extended Spectrum B-Lactamase (ESBL) Producing *Escherichia coli* and Molecular Characterization of ESBL, Carbapenemases, and BlaCMY2 Genes in Broilers and Humans at Menoufia Governorate, Egypt

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**ABSTRACT**

Antibiotic resistance is a serious worldwide problem threatening human health and life. Poultry, especially broilers, represent a major source of ESBL-producing *E. coli* due to the extensive use of antibiotics during their production. This study investigated the prevalence of ESBL-EC in broilers, the surrounding environment, and humans, as well as the occurrence of ESBLs, carbapenemases, and bla\(^{\text{CMY2}}\) genes. 249 samples were included in that study: 130 broiler cloacal swabs, 54 environmental samples, and 65 human samples. All samples were grown on MacConkey broth, then cultured on EMB containing cefotaxime sodium. Suspected *E. coli* isolates were identified and confirmed biochemically by IMVIC tests. The disc diffusion method was used to determine the antibiotic susceptibility pattern of *E. coli* isolates grown on EMB with cefotaxime. PCR was performed for the amplification of ESBLs, carbapenemases, and bla\(^{\text{CMY2}}\) genes. Our results revealed that ESBL-EC was isolated from 180 (72.3%) of the 249 collected samples. The prevalence of ESBL-producing *E. coli* was as follows: 85.4%, 46.3%, and 67.7% in broiler cloacal swabs, environmental samples, and human samples, respectively. Among 188 *E. coli* isolates grown on EMB containing cefotaxime, 187 were resistant to CTX, 170 to CAZ, 166 to FEP, and 146 to FOX. PCR amplification showed that the predominant gene was bla\(^{\text{TEM}}\), which was detected in 98% of the amplified isolates, followed by bla\(^{\text{CTX-M}}\) (88%). Bla\(^{\text{KPC}}\) wasn't detected in any isolates. Many gene combinations were detected, and the most common one was bla\(^{\text{TEM}}\) with bla\(^{\text{CTX-M}}\), which was detected in 24 (48%) of the 50 isolates.

**Keywords:** Broiler, Carbapenemases, *E. coli*, ESBLs and Human.

**INTRODUCTION**

Antibiotics are prescribed mainly for the treatment of bacterial infections and prophylaxis of diseases (FAO/WHO/OIE, 2008). However, antibiotics use may be a main factor in developing and spreading of antibiotic resistance in normal bacterial flora and target pathogens. Consequently, antibiotic-resistant bacteria have an increasing surge, globally (EFSA, 2009). Zoonotic antimicrobial-resistant bacteria such as *E. coli* have many consequences, such as failure to treat human infections and prolongation of treatment periods more than usual. WHO identifies cephalosporins and fluoroquinolones as essential...
antibiotics for the treatment of human infections. Additionally, improper abuse and/or misuse in the agricultural and animal production sectors should not compromise the effectiveness of critical antibiotics for use in treating humans (FAO/WHO/OIE, 2008). But among bacteria of zoonotic importance, the rate of resistance to therapeutically significant antibiotics including carbapenems, Extended spectrum-lactams, aminoglycosides, and fluoroquinolones has increased (Bitrus et al., 2019).

Poultry industry is a vast global food industry. Chickens dominate global farmed animal production, producing over 90 billion tons annually (FAO, 2017). The extensive use of antibiotics in broiler production enhances the evolution of bacteria resistant to antibiotics (Diarra & Malouin, 2014). In addition, poultry has been implicated as a possible source of ESBL-producing bacteria that can infect humans and cause intestinal colonization and serious illness when transmitted to humans via direct contact or ingestion of contaminated meat and meat products (Lazarus et al., 2015).

*E. coli* is regularly found in food, water, and soil due to faecal contamination or contamination during the slaughter of food animals. It is also frequently found in the gastrointestinal tracts of both humans and animals. Several *E. coli* strains cause colibacillosis in poultry, and some of which are responsible for severe diseases in humans like hemorrhagic colitis and hemolytic uremic syndrome (Ferens & Hovde, 2011). *E. coli* is present in both humans and animals as a part of intestinal commensal flora. *E. coli* and other bacteria found in the commensal flora can act as a reservoir for antibiotic resistance genes spreading them to other bacterial species, including those that can infect both people and animals. The consequences of antibiotic usage and changes in the prevalence of antimicrobial resistance in food-producing animals should be studied more thoroughly in these indicator bacteria rather than in food-borne pathogens (EFSA, 2008).

Extended-spectrum β-lactamases (ESBLs) are a group of enzymes carried on plasmid and provide resistance to cephalosporins, and inhibited by clavulanic acid (Bush & Jacoby, 2010). ESBLs are regarded as troublesome on a global scale and are becoming more common in various regions of the world (Ghafourian et al., 2015). A variety of Gram-negative bacteria have the intrinsic beta-lactamase enzyme AmpC on their chromosomes. With the exception of carbapenem and fourth-generation cephalosporins, they are effective against cefoxitin, penicillin, cephalosporins, cephamycin, and β-lactam/inhibitor combination (Carattoli, 2008). Carbapenemases are β-lactamases with a variety of hydrolytic capabilities. Penicillins, cephalosporins, monobactams, and carbapenems can all be destroyed by them. Bacteria that produce these β-lactamases can cause harmful infections in which the action of numerous β-lactams renders them useless (Queenan & Bush, 2007).

ESBL-EC colonization was found to be enhanced in both healthy humans and animals globally due to the haphazard use of antibiotics in both populations. It was proposed that animals, particularly poultry, might transmit bacterial genetic components carrying ESBL-encoding genes and resistant plasmids to humans (Nguyen et al., 2019).

In Egypt, a study made on poultry and poultry products concluded that all *E. coli* isolates had ESBL encoding genes. PCR revealed that blaTEM was found in 100% of the *E. coli* isolates tested but blaSHV was only found in 10% of them. All of the isolates were devoid of the blaCTX-M gene (Kamel et al., 2021). Furthermore, in another study...
carried out on humans with infections of urinary tract, a total of 80 (59.7%) out of 134 uropathogenic E. coli (UPEC) investigated were reported to produce ESBLs. Sixty-two percent (62%) of ESBL producers were multi-drug resistance (MDR). They were all sensitive to meropenem. The Most prevalent genes were blaTEM (60%) and blaCTX-M (45%) (Hassuna et al., 2020).

Broiler faecal matter contain E. coli strains that can grow and integrate into a model of the human digestive system. Plasmids bearing ESBL genes can simultaneously spread from the E. coli poultry strain to strains of E. coli with human origin (Smet et al., 2011). Sadly, Egypt lacks legislation regulating antibiotic use, with some antibiotics like tetracycline, quinolones, and beta-lactams still used for other purposes rather than therapeutic ones (WHO, 2014). Improper antimicrobial use in poultry leads to high evolution of multi-resistant Enterobacteriaceae strains (Moawad et al., 2017).

Based on clinical microbiology techniques, many ESBL detection approaches have been proposed. These methods comprise preliminary screening for ESBL generation followed by confirmation testing. Disk diffusion methods are frequently used for screening, whereas phenotypic confirmatory methods primarily rely on the enhancement of the inhibitory zone caused by beta-lactamase inhibitors such as clavulanic acid and tazobactam (Salihu et al., 2020).

It is still advised to use carbapenems (imipenem, meropenem, ertapenem, doripenem) for the treatment of severe infections caused by ESBL-EC and K. pneumoniae. According to reports, over 98% of ESBL-EC respond to these medications (Perez et al., 2007).

So, this study inspected the prevalence of ESBL-EC in broilers, farms and retail poultry shop workers, and community humans, and identified ESBLs, carbapenemases, and CMY2 encoding genes in resistant E. coli strains.

**MATERIAL AND METHODS**

**Study site and sample collection:**

The study was made in El Shuhada district, Menofia governorate, for six months, from June 2022 to November 2022. Samples were collected from 3 broiler farms, 10 retail poultry shops, and 3 human clinical laboratories. A total No. of 249 samples were collected, including 130 broiler cloacal swabs (80 from broiler farms and 50 from retail poultry shops), environmental samples (54), and human samples (65). The age of broilers at the time of sample collection was between 30 and 35 days at the end of the fattening period. Different types of environmental samples were collected as follows: 12 litter samples, 12 feeding trough swabs, and 12 watering trough swabs from broiler farms, and 18 working stage swabs from retail poultry shops. Collected human samples were divided into hand swabs (18) obtained from broiler farms and retail poultry shop workers and stool swabs (47) obtained from human clinical laboratories. Swab samples were collected using sterile cotton swabs, inserted into saline tubes, and transferred to the laboratory for examination.

**Isolation and identification of ESBL producing E. coli:**

Firstly, all samples were grown on MacConkey broth (Oxoid, UK) for
enrichment. Yellow colouration and gas formation in macConkey broth tubes indicate the presence of coliforms. Then, a loopful from yellow MacConkey broth tubes were streaked onto eosin methylene blue agar (EMB) (TM MEDIA, India) containing 2 mg/l cefotaxime sodium (MERCK, Germany) and incubated at 37°C for 18:24 hrs. Suspected *E. coli* colonies were confirmed biochemically using indole, methyl red, vogues Proskauer, citrate utilization, and triple sugar iron tests. Typical *E. coli* colonies were subcultured on glycerol broth 20% and stored at -20°C for subsequent study.

**Antimicrobial susceptibility testing:**

Kirby-Bauer disk diffusion method was used for determination of antimicrobial susceptibility pattern of potential ESBLs producing *E. coli* isolates using several antibiotic discs including cefotaxime (CTX, 30 µg), ceftazidime (CAZ, 30 µg), cefepime (FEP, 30 µg), cefoxitin (FOX, 30 µg), meropenem (MEM, 10 µg), and imipenem (IPM, 10 µg) (Bioanalyse, Turkey). The test was carried out according to EUCAST Disk Diffusion Method for Antimicrobial Susceptibility Testing. The measured zone diameters were recorded, and compared to guideline zone diameters recommended by CLSI.

**Phenotypic confirmation of ESBL producers:**

ESBL production was confirmed by a combination disc test (CDT), recommended by CLSI. Cefotaxime and ceftazidime with and without clavulanic acid discs were applied on inoculated MHA surface with a 20 mm distance between the discs and then plates incubated at 37°C for 18 hrs. ESBL-positive isolates were identified by a zone diameter increase of 5mm or more for either antimicrobial agents combined with clavulanic acid in comparison to zone diameter when tested alone (CLSI, 2022).

**Molecular detection of ESBLs genes, carbapenemases genes and blaCMY2 gene:**

A No. of 50 resistant isolates were selected for detection of genes responsible for resistance. G-spin Total DNA Extraction Kit (iNtRON Biotechnology, Korea) was used for DNA extraction and the protocol was carried out according to the kit manual. PCR amplification was performed by certain oligonucleotide primers to detect resistance-encoding genes; TEM, SHV, CTX-M, OXA48, NDM, KPC, and CMY2 (Table 1). Amplification of target genes was done using Easy Taq DNA polymerase (TRANSGEN biotech, China) where each isolate was subjected to three PCR reactions as shown in (Table 2). Agarose gel electrophoresis was used to determine the size of amplified genes using a 100 bp plus DNA ladder (TRANSGEN biotech, China).

### Table 1: Oligonucleotide primers used for PCR amplification of β-lactamase genes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence 5’-3’</th>
<th>Product size, pb</th>
<th>Reference</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>blaTEM</strong></td>
<td>TCGCCGCATACACTATTCTCAGAAATGA</td>
<td>445</td>
<td>Monstein et al. (2007)</td>
<td>Metabolic AG (Germany)</td>
</tr>
<tr>
<td></td>
<td>ACGCTCACC GGCTCAGATTTAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>blaSHV</strong></td>
<td>ATGCGTTATATTTCGCTGTG</td>
<td>747</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TGCTTTTATTTCGGGCAA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Amplification conditions of each PCR reaction:

<table>
<thead>
<tr>
<th>Target resistance genes</th>
<th>TEM, CTX-M, SHV(^{(1)})</th>
<th>OXA48, NDM, KPC(^{(2)})</th>
<th>CMY2(^{(1)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final volume</td>
<td>25 µl</td>
<td>25 µl</td>
<td>25 µl</td>
</tr>
<tr>
<td>Initial denaturation</td>
<td>95°C (15 min)</td>
<td>94°C (10 min)</td>
<td>94°C (5 min)</td>
</tr>
<tr>
<td>Denaturation</td>
<td>94°C (30 s)</td>
<td>94°C (30 s)</td>
<td>94°C (1 s)</td>
</tr>
<tr>
<td>Annealing</td>
<td>60°C (30 s)</td>
<td>52°C (40 s)</td>
<td>61°C (1 s)</td>
</tr>
<tr>
<td>Extension</td>
<td>72°C (2 min)</td>
<td>72°C (50 s)</td>
<td>72°C (1 min)</td>
</tr>
<tr>
<td>Final extension</td>
<td>72°C (10 min)</td>
<td>72°C (5 min)</td>
<td>72°C (5 min)</td>
</tr>
</tbody>
</table>

\(^{(1)}\) 30 cycles of denaturation, annealing, and extension; \(^{(2)}\) 36 cycles of denaturation, annealing, and extension

RESULTS

ESBL-EC was determined in 180 (72.3%) of the 249 collected samples. The prevalence of ESBL-EC obtained from broiler cloacal swabs was 85.4%. Regarding farms, the within-farm prevalence in cloacal swabs was; 96% for farm 1, 80% for farm 2 and 90% for farm 3 (Table 3).

Table 3: Prevalence of ESBL-EC in broiler cloacal swabs collected from both farms and retail poultry shops.

<table>
<thead>
<tr>
<th>No. of examined samples</th>
<th>Positive ESBL-EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
</tr>
</tbody>
</table>
Moreover, the prevalence of ESBL-EC in different environmental samples was 46.3% which is described as follows; 58.3%, 50%, 41.7, and 38.9% in litter, feeding trough swabs, watering trough swabs and working stage swabs, respectively (Table 4).

**Table 4**: Prevalence of ESBL-EC in different environmental samples.

<table>
<thead>
<tr>
<th>No. of examined samples</th>
<th>Positive ESBL-EC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Litter</td>
<td>12</td>
</tr>
<tr>
<td>Feeding trough swabs</td>
<td>12</td>
</tr>
<tr>
<td>Watering trough swabs</td>
<td>12</td>
</tr>
<tr>
<td>Working stage swabs</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
</tr>
</tbody>
</table>

With reference to human samples, ESBL-EC was isolated from 44 (67.6%) of 65 collected samples. Two forms of human samples were collected; hand swabs and stool swabs having a prevalence of 38.9% and 78.7%, respectively (Table 5).

**Table 5**: Prevalence of ESBL-EC in human swab samples.

<table>
<thead>
<tr>
<th>No. of examined samples</th>
<th>Positive ESBL-EC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Hand swabs</td>
<td>18</td>
</tr>
<tr>
<td>Stool swabs</td>
<td>47</td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
</tr>
</tbody>
</table>

Among 188 *E. coli* isolates growing on EMB containing cefotaxime, all *E. coli* isolates from different samples had a more or less similar resistance trend, where 187 isolates were resistant to CTX, 170 to CAZ, 166 to FEP, 146 to FOX, 1 to MEM, and 1 to IPM (Table 6). The resistant isolate to meropenem and imipenem was obtained from human stool sample.

**Table 6**: Antimicrobial resistance pattern of the 188 *E. coli* isolates growing on EMB containing cefotaxime.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Cloacal swabs (n=115) No. (%)</th>
<th>Environmental samples (n=27) No. (%)</th>
<th>Human samples (n=46) No. (%)</th>
<th>Total (n=188) No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotaxime (CTX) 30 µg</td>
<td>115 (100%)</td>
<td>27 (100%)</td>
<td>45 (97.8%)</td>
<td>187 (99.5%)</td>
</tr>
<tr>
<td>Ceftazidime (CAZ) 30 µg</td>
<td>101 (87.8%)</td>
<td>25 (92.6%)</td>
<td>44 (95.7%)</td>
<td>170 (90.4%)</td>
</tr>
<tr>
<td>Cefepime (FEP) 30 µg</td>
<td>105 (91.3%)</td>
<td>22 (81.5%)</td>
<td>39 (84.8%)</td>
<td>166</td>
</tr>
</tbody>
</table>
Combination disc test revealed that 180 E. coli isolates out of 188 potential ESBL producing strains, were ESBL producers, while the remaining 8 strains were non-ESBL producers. All the eight non-ESBL producing confirmed isolates were resistant to cefoxitin.

Among the 50 E. coli isolates subjected to PCR amplification, blaTEM was detected in 49 isolates, blaCTX-M in 44 isolates, blashv in 8 isolates, blacMY2 in 13 isolates, blaoXA48 in 10 isolates, and blanDM was detected only in one isolate obtained from human stool (Table7). No isolate was containing blakPC gene. Many gene combinations were detected and the most common one was blatem with blactx-M which occur in 24 (48%) of the 50 isolates (Figure 2).

**Table 7:** Occurrence of ESBL genes, carbapenemases genes and blacMY2 gene in genotypically screened E. coli isolates.

<table>
<thead>
<tr>
<th>Detected gene</th>
<th>Cloacal swabs (n=26) No. (%)</th>
<th>Environmental samples (n=7) No. (%)</th>
<th>Human samples (n=17) No. (%)</th>
<th>Total (n=50) No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefoxitin (FOX) 30 µg</td>
<td>87 (75.7%)</td>
<td>20 (74%)</td>
<td>39 (84.8%)</td>
<td>146 (88.3%)</td>
</tr>
<tr>
<td>Meropenem (MEM) 10 µg</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (2.2%)</td>
<td>1 (0.5%)</td>
</tr>
<tr>
<td>Imipenem (IPM) 10 µg</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (2.2%)</td>
<td>1 (0.5%)</td>
</tr>
</tbody>
</table>

1Percentage of resistant isolates.
Figure 2: Occurrence of ESBL genes, carbapenemases genes and blaCMY2 gene combinations in genotypically screened *E. coli* isolates.

<table>
<thead>
<tr>
<th></th>
<th>TEM</th>
<th>CTX-M</th>
<th>SHV</th>
<th>CMY2</th>
<th>OXA48</th>
<th>NDM</th>
<th>KPC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>26 (100%)</td>
<td>7 (100%)</td>
<td>16 (94.1%)</td>
<td>49 (98%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>24 (92.3%)</td>
<td>6 (85.7%)</td>
<td>14 (82.4%)</td>
<td>44 (88%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 (19.2%)</td>
<td>1 (14.3%)</td>
<td>2 (11.8%)</td>
<td>8 (16%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9 (34.6%)</td>
<td>3 (42.9%)</td>
<td>1 (5.9%)</td>
<td>13 (26%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 (19.2%)</td>
<td>1 (14.3%)</td>
<td>4 (23.5%)</td>
<td>10 (20%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (5.9%)</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1Percentage of positive isolates.

Figure 3: Prevalence of resistance genes in *E. coli* obtained from cloacal swabs and environmental samples in comparison to isolates from humans: A, cloacal swabs; B, environmental samples; C, humans.
Figure 4: Agarose gel electrophoresis of multiplex PCR of ESBL genes in *E. coli* isolates; Lane M: 100bp plus DNA Marker (100:1500bp); Lane No. 4 is positive for *bla*TEM, *bla*SHV and *bla*CTX-M; Lanes No. 2, 3, 5, 7, 8, 9 and 10 are positive for *bla*TEM and *bla*CTX-M; Lane No. 6 is positive for *bla*TEM; Lane No. 1 is negative.

Figure 5: Agarose gel electrophoresis of uniplex PCR of CMY2 gene in *E. coli* isolates; Lane M: 100bp plus DNA Marker (100:1500bp); Lanes No. 3, 5, 6, 8, 9, 10, 11 and 12 are positive for *bla*CMY2; Lanes No. 1, 2, 4, 7, 13, 14 and 15 are negative.
DISCUSSION

ESBL-EC in poultry poses a public health concern as it makes treatment difficult with commonly used β-lactam antibiotics, increasing the risk of death and preventing proper treatment. Although cephalosporins and penicillin/β-lactamase inhibitors may be effective, they are rarely used as first-choice therapy (Chong et al., 2018).

In the present study, ESBL-EC was detected in 180 (72.3%) of the 249 collected samples. This high prevalence may be attributed to many factors such as the overuse of antibiotics during the rearing period of broilers as co-selection under antibiotic pressure was discussed as a potential reason for the enrichment of ESBL/AmpC-producing *E. coli* on broiler fattening farms (Costa et al., 2009; Dierikx et al., 2013; Smet et al., 2010). Also, the lack of hygienic practices and biosecurity measures inside the investigated farms as well as the persistence of resistant bacteria inside poultry houses due to inadequate cleaning and disinfection which also was reported by Hiroi et al. (2012). Moreover, the misuse of antibiotics by humans and bad personal hygiene and many farm workers may work on different farms may be effective factors increasing the prevalence of ESBL-EC.

The prevalence of ESBL-EC in broiler cloacal swabs was 85.4% and that finding is consistent with other studies conducted in Egypt (82.2%) (Ahmed, 2015), Belgium (85%) (De Koster et al., 2021), and China (88.8%) (Li et al., 2016). In a study made in Netherlands, it was found that 85% of examined broiler farms had a prevalence of 80% or higher (Dierikx et al., 2013). Also, a prevalence of 96.4% of ESBL-EC was found in Dutch broiler farms (Huijbers et al., 2014). In a study conducted on broiler farms and hospitalized children in Ghana, the prevalence of ESBL-EC in broilers was 29% (Falgenhauer et al., 2019). Moreover, a study made in Luzon, Philippines reported that the prevalence of ESBL-EC in pooled broiler cloacal swabs was 60.26% (Gundran et al., 2019). This clearly demonstrates that the prevalence of ESBL-producing *E. coli* varies by country.

In environmental samples, the overall prevalence of ESBL-EC was 46.3%: 58.3% in litter samples, 50% in feeding trough swabs, 41.7% in watering trough swabs, and 38.9% in working stage swabs. In a previous study made at broiler chicken fattening farms, ESBL-EC was detected in all examined environmental samples with a prevalence of 54.2% in all environmental swab samples and 100% in litter samples.
The detection of ESBL-EC in the environment in this study suggests the potential spread of the pathogen in the environment, which may have a role in the transmission to farm workers, retail poultry shop workers, and the general public, as formerly reported (Bui et al., 2018; Huijbers et al., 2014).

With reference to humans, we found a high prevalence (78.7%) of ESBL-EC in stool swabs collected from human clinical laboratories, while in hand swabs from broiler farms and retail poultry shop workers, the prevalence was 38.9%; these findings show that there is a rise in the human faecal ESBL-EC carriage in the Egyptian community from 45.1% (Abdul Rahman & El-Sherif, 2011) to 78.7% in our study which is in harmony with other studies accomplished in Tanzania (76.3%) (Büdel et al., 2019), and Vietnam (75.1%) (Thi Quynh Nhi et al., 2018).

In antimicrobial susceptibility testing, 99.5% of E. coli isolates were resistant to cefotaxime followed by 90.4% to ceftazidime, 88.3% to cefepime, and 77.7% to cefoxitin. A study made in China on healthy broilers showed some similarity to our results where 98.6% of isolates were resistant to cefotaxime, 95.1% to cefepime, and in contrast to our results, 32.4% were resistant to ceftazidime (Li et al., 2016). Interestingly and in contrary to our findings, a study performed in Egypt reported resistance percents of 73.8%, 52.3%, and 35.4% to cefotaxime, ceftazidime, and cefepime, respectively (Badr et al., 2022).

Regarding carbapenems, all 126 E. coli isolates from both cloacal and environmental samples were sensitive to meropenem and imipenem and this was similar to other previous studies in Egypt (Badr et al., 2022), China (Li et al., 2016), as well as Belgium and Netherlands (De Koster et al., 2021).

Among the 50 selected isolates for PCR amplification, blaTEM was the most prevalent gene in 98% of the examined isolates, followed by blaCTX-M in 88%. A study conducted in Egypt on poultry and poultry products agrees with our results, as blaTEM was found in 100% of E. coli isolates (Kamel et al., 2021). Also, in contrast to our study, Badr et al. (2022) reported a high incidence of blaCTX-M followed by blaTEM in E. coli obtained from chicken farms in Egypt. For humans, it was reported that blaTEM was the predominant gene in studies from the Philippines (Cruz & Hedreyda, 2017), and Saudi Arabia (Hemeg, 2018). Other studies made in Egypt (Badr et al., 2022), Germany (Dahms et al., 2015), Netherlands (Overdevest et al., 2011) mentioned that blaCTX-M gene was the most common gene detected in E. coli strains isolated from humans.

Many gene combinations were detected in this study and the most frequent one was blaTEM/blaCTX-M in 24 (48%) from 50 E. coli isolates which agrees with previous studies detecting ESBL genes in poultry cloacal swabs (Gundran et al., 2019; Khoshbakht et al., 2016; Li et al., 2016). Also in Kenya, it was found that blaTEM/blaCTX-M (69.6%) was the commonly found gene combination in uropathogenic E. coli obtained from humans (Muriuki et al., 2022). Multiple ESBL resistance genes may give a chance for the maintenance of β-lactamase resistance although the diminished expression of one or two genes (Gundran et al., 2019).

Interestingly, one E. coli isolate was phenotypically confirmed as ESBL producer with no TEM, CTX-M, or SHV gene detected on PCR and that is in harmony with a study made in Germany that confirmed 6 E. coli isolates as ESBL producers without TEM, CTX-M, or SHV genes detected in these six isolates (Laube et al., 2013).
In recent study, the most commonly found carbapenemase gene in broiler cloacal swabs and their surrounding environment was bla\text{OXA48} while bla\text{NDM} and bla\text{KPC} weren’t detected in any isolate. These findings are consistent with a previous study performed in Malaysia on \textit{E. coli} strains harboring carbapenemases genes (Aklilu et al., 2020). Also, in a study made in France and Algeria for detection of carbapenemases genes in broiler feces, no bla\text{OXA48}, bla\text{NDM}, or bla\text{KPC} was detected (Chabou et al., 2018). Regarding humans, bla\text{OXA48} was the predominant carbapenemase encoding gene which agrees with a preceding study made in Turkey (Kizilates et al., 2021). Additionally, in contrast to our findings, it was reported that NDM gene was the most common carbapenemase gene in Iran (Hajihasani et al., 2022), and Shanghai (Pan et al., 2019). Co-occurrence of OXA48 gene with ESBL genes was detected especially TEM (9 isolates) and CTX-M (7 isolates) which is in harmony with previous studies stated that OXA-48 was found in ESBL producing strains (Mushi et al., 2014; Potron et al., 2013). It is worth mentioning that eight isolates among ten OXA48 harbouring isolates were sensitive to imipenem and meropenem. This was also reported in a previous study made in Tanzania (Mushi et al., 2014), and in a study carried out by Potron et al. (2013) where 65% of OXA48 harbouring isolates were susceptible to meropenem and imipenem, consequently complicating the detection of OXA-48 producing isolates in laboratories.

The bla\text{CMY2} gene was detected mainly in broiler cloacal swabs and environmental samples (12 isolate), while in humans was detected only in one isolate. A previous study made on Dutch broiler farms and farm workers agrees with our results, where bla\text{CMY2} gene was found in 12, and 5 broiler and farmer isolates, respectively (Dierikx et al., 2013).

CONCLUSIONS
From that study, we concluded that there is a high prevalence of ESBL-EC in broiler farms, and the surrounding environment, as well as in humans either in contact with broilers or other community humans. Seriously, these resistant bacteria can be transmitted zoonotically from broilers and their environment to humans which endangers human health by making the treatment of human infections more difficult with routine antibiotics.

DECLARATION
The authors have no competing interests.

ETHICAL APPROVAL
The study was approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, University of Sadat City (VUSC-045-1-22).

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