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# Molecular Detection of *blaTEM, blaSHV* and *blaOXA* from *Escherichia Coli* Isolated from Chickens

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## Abstract

Colibacillosis is considered as one of the major avian pathogens. This work is conducted to determine the extended spectrum β-lactamase (ESBL) producing *Escherichia coli* (*E. coli*) isolated from chickens, where intensive drug use is common. A total of 344 samples including; liver, heart, spleen and lung were collected separately from 86 diseased birds (alive and freshly dead chickens), (1 to 40) days old. For the presence of β-lactamase encoding genes (*blaTEM, blaSHV*, and *blaOXA*), 112 (32.5%) out of those samples was *E. coli* positive. The prevalence of *E. coli* in the different organs was as the following: liver 44.1% (38/86), heart 30.2% (26/86), spleen 29% (25/86) and lung 26.7% (23/86). The commonly identified *E. coli* serogroups were O125, O127, O27, O29, O86, O112, O148, and finally O20 and O157. This shows multidrug-resistant to a variety of antibiotics, (100%) of resistance was observed with Pencillins, Tetracycline, Vacomycin and Erythromycin. (50%) of resistance to Chloramphenicol, Cefotaxime and Nalidixic acid. (0%) resistance to Amikacin and Gentamycin. Depending on the results of PCR, the *Stx1*and *eaeA* was negative in all the isolates. Only one isolate O112 carried the *Stx2* gene, *blaTEM* was the most predominant gene (100%), followed by *blaSHV* (22%), then, *blaOXA* had the lowest prevalence (11%). This results show that prevalence of β-lactamase encoding genes of *E. coli* in diseased chickens which related to resistance against beta-lactam antibiotics.

**Keywords:** *blaTEM; blaSHV; blaOXA;* chickens; *E. coli*

## Introduction

Egypt is listed among the countries with high prevalence of antimicrobial resistance, especially extended-spectrum β-lactamases (ESBLs) [1]. Wide spread use of broad spectrum beta lactams as antimicrobial therapy leads to induction of ESBLs production in *Escherichia coli* (*E. coli*) which resulted from mutations in the genes of common plasmid mediates beta-lactams specially TEM (Temoneira) and SHV (sulfhydryl variable). This produces alteration in the enzyme configuration and increased affinity and hydrolytic ability of beta lactamase [2]. Pathogenic bacteria, including *E. coli*, that produce ESBL show resistance to diversity of β-lactams and some non-β-lactam drugs including aminoglycosides, fluoroquinolones and sulphamethoxazole [3]. Almost all the (ESBL) genes are carried by mobile genetic elements of *E. coli*, and the genes can spread both clonally and horizontally among different lineages of *E. coli* [4]. (ESBLs) are plasmid-encoded enzymes found in Gram-negative bacteria especially in *Enterobacteriaceae* gives resistance to first, second and third generation cephalosporins while they are inhibited by clavulanic acid [5]. The primary and secondary environments of *E. coli* are intestinal tract of warm-blooded animals. In poultry, *E. coli* lives in the lower digestive tract where colonizes it in the first 24 h after hatching [6]. *E.coli* is Gram-negative bacteria found in the environment, intestinal tracts of animals and humans. Most *E. coli* strains are harmless and are an important part of a healthy animal and human intestinal tract. However, some strains of *E. coli* have acquired virulence features and are called pathogenic *E.coli* [7]. The commensal *E. coli* in chicken gut improve resistance either due to chromosomal mutation or the acquisition of resistance features from mobile genetic elements (e.g. transposons, plasmids and integrons) [8]. Antimicrobial resistance is one of the global threats that impact animal and human health especially in developing countries [9]. *E. coli* encoding for ESBL and carbapenemases are resistant to more than one class of antimicrobials and subsequently are multidrug-resistant (MDR). In addition, effects of antimicrobials for prophylaxis and as growth promoters, resulting in emergence and development of resistance against these compounds [10].

The purpose of this study is to determine the antibiotic resistance and highlights the relationship between phenotypic and genotypic resistance of pathogenic *E. coli* isolates. So must be applying a nationwide surveillance program to monitor antimicrobial resistance where most of administration of these antimicrobials is unnecessary.

## Materials and Methods

### Sample collection

A total of 344 tissue specimens including (86) liver, (86) heart, (86) spleen, and (86) lung were collected separately from 86 broiler chickens (43 from freshly dead and 43 from diseased alive), 1 to 40 days old, intensive production system, from different farms in Aswan governorate, Egypt. The samples were subjected to bacteriological analysis.

### Bacteriological examination

A sample of 25 g from each chicken organ was homogenized in 225 ml of Puffer Peptone Water (PPW) and incubated at 37 °C for 18-24 h, according to Quinn, *et al.* [11]. After incubation, a loopful from inculated (PPW) was seeded onto MACCONKEY'S AGAR (Oxoid) plates for 24 h at 37 °C. Rose pink colonies were picked up and streaked onto EOSIN METHYLENE BLUE AGAR (Oxoid) and incubated overnight at 37 °C. Green metallic sheen colonies of *E. coli* were picked up for biochemical tests, Indole, Triple Sugar Iron Agar, Citrate utilization, Methyl Red-Voges-Proskauer and Urease production tests.

### Serological typing of *E. coli*

It was performed according to Edwards and Ewing [12]. Serotyping of *E. coli* isolates were done at the Reference Laboratory of Veterinary Quality Control on Poultry Production, Dokki, Egypt using commercially available kits (*Escherichia coli* Antisera set 1 for O antigen, DENKA SEIKEN, Tokyo, Japan) which consists of 8 polyvalent sera and 43 monovalent sera.

### Antibacterial Sensitivity test

The disk diffusion method was applied Kirby-Bauer method according to Cruickshank *et al.* [13]. Mueller Hinton broth (Oxoid) is used in preparation of inoculums in antimicrobial susceptability test, Mueller Hinton agar (Oxoid) used for inoculation of isolates where antibiotic discs are placed on the surface of the agar to diffuse into the medium creating a halo zone of inhibition around the antimicrobial disc. The zones of inhibition were measured in millimeters using a ruler. The interpretation of inhibition zones of tested culture was according to CLSI.

Selected twelve antibiotic discs including Penicillin G(10µg), Amoxicillin/Clavulanic(30µg), Chloramphenicol(30µg), Erythromycin(15µg), Cefotaxime(30µg), Amikacin(30µg), Gentamycin(10µg), Sulphamethoxazole/Trimethoprim(1.25-23.75µg), Tetracycline(30µg), Nalidixic acid(30µg), Vancomycin(30µg) and Norfloxacin(10µg) (Oxoid).

### Molecular detection of virulence genes and ESBL encoding genes

Multiplex PCR was applied for detection of virulence genes involving Shiga toxin 1 (*Stx1*), Shiga toxin 2 (*Stx2*) and intimin (*eaeA*) genes as well as (ESBL) genes *blaTEM*, *blaSHV* and *blaOXA*.

### Extraction of *DNA*

According to QIA amp DNA Mini Kit instructions (Catalogue no.51304) that provides silica-membrane-based nucleic acid purification from different types of samples. The spin-column procedure does not require mechanical homogenization, so total hands-on preparation time is only 20 minutes. They have specific sequence of primers and amplify specific products as shown in Table 1.

| Gene | Primer sequence (5'-3') | Product | Reference |
|---|---|---|---|
| ***blaTEM*(F)** | ATCAGCAATAAACCAGC | 516 bp | Colom, *et al.* (2003) |
| ***blaTEM*(R)** | CCCCGAAGAACGTTTTC | | |
| ***blaSHV*(F)** | AGGATTGACTGCCTTTTTG | 392 bp | |
| ***blaSHV*(R)** | ATTTGCTGATTTCGCTCG | | |
| ***blaOXA-1*(F)** | ATATCTCTACTGTTGCATCTCC | 619 bp | |
| ***blaOXA-1*(R)** | AAACCCTTCAAACCATCC | | |
| ***eaeA*(F)** | ATG CTT AGT GCT GGT TTA GG | 248 bp | Bisi-Johnson, *et al.* (2011) |
| ***eaeA*(R)** | GCC TTC ATC ATT TCG CTT TC | | |
| ***Stx1*(F)** | ACACTGGATGATCTCAGTGG | 614 bp | Dipineto, *et al.* (2006) |
| ***Stx1*(R)** | CTGAATCCCCCTCCATTATG | | |
| ***Stx2*(F)** | CCATGACAACGGACAGCAGTT | 779 bp | |
| ***Stx2*(R)** | CCTGTCAACTGAGCAGCACTTTG | | |

**Table 1:** Oligonucleotide primer sequences

## PCR Master Mix used for cPCR

Emerald Amp GT PCR master mix (Takara) Code No. RR310A.

## Cycling conditions of the primers during cPCR

Temperature and time conditions of the primers during PCR are shown in Table 2.

| Gene | Primary denaturation | Secondary denaturation | Annealing | Extension | No.of cycles | Final extension |
|---|---|---|---|---|---|---|
| ***blaTEM*** | 94 °C<br>5 min | 94 °C<br>30 sec | 54 °C<br>40 sec | 72 °C<br>45 sec | 35 | 72 °C<br>10 min |
| ***blaSHV*** | 94 °C<br>5 min | 94 °C<br>30 sec | 54 °C<br>40 sec | 72 °C<br>40 sec | 35 | 72 °C<br>10 min |
| ***blaOXA-1*** | 94 °C<br>5 min | 94 °C<br>30 sec | 54 °C<br>40 sec | 72 °C<br>45 sec | 35 | 72 °C<br>10 min |
| ***eaeA*** | 94 °C<br>5 min | 94 °C<br>30 sec | 51 °C<br>30 sec | 72 °C<br>30 sec | 35 | 72 °C<br>7 min |
| ***Stx1, Stx2*** | 94 °C<br>5 min | 94 °C<br>30 sec | 58 °C<br>40 sec | 72 °C<br>45 sec | 35 | 72 °C<br>10 min |

**Table 2:** Cycling conditions of the primers during cPCR

## DNA Molecular weight marker

The ladder was mixed gently by pipetting up and down 6 µl of the required ladder was directly loaded.

## Agarose gel electrophoreses [16]

Electrophoresis grade agarose (1.5 g) was prepared in 100 ml TBE buffer in a sterile flask, it was heated in microwave to dissolve all granules with agitation, and allowed to cool at 70 °C, then 0.5µg/ml Ethedium bromide was added and mixed thoroughly.

The gel was photographed by a gel documentation system and the data was analyzed through computer software.

# Results

## Incidence of *E. coli* infection in chicken

From total of 344 organ samples there were 112 (32.5%) *E. coli* positive. The highest percentages of organs isolation were obtained from Liver 38/86 (44.1%), followed by Heart 26/86 (30.2%), Spleen 25/86 (29%), and finally Lung 23/86 (26.7%).

## Serotyping of *E. coli* isolates recovered from chicken samples

The serotyping of *E. coli* strains isolated from different organs of chickens (78%) belonged to (9) different 'O' groups, while (22%) strains were untypeable by available antisera.

The most commonly detected *E. coli* serogroups isolated from different organs of chickens were O125 (28%), O127 (12%), O27 (8%), O29 (8%), O86 (7.5%), O112 (7.5%), O148 (7.5%), O20 (2%) and O157 (2%).

## Antibiotic sensitivity of *E. coli* strains

Multidrug resistance was detected in all isolated serotypes (100%) of resistance was observed with Pencillins, Tetracycline, Vancomycin, and Erythromycin antibiotics. Followed by Chloramphenicol, Cefotaxime and Nalidixic acid were resistance (50%), Sulphamethoxazole / Trimethoprim (40%), Norfloxacin (30%), Amoxicillin/ clavulanic acid (20%), and Amikacin and Gentamycin 0% to all isolates Table 2.

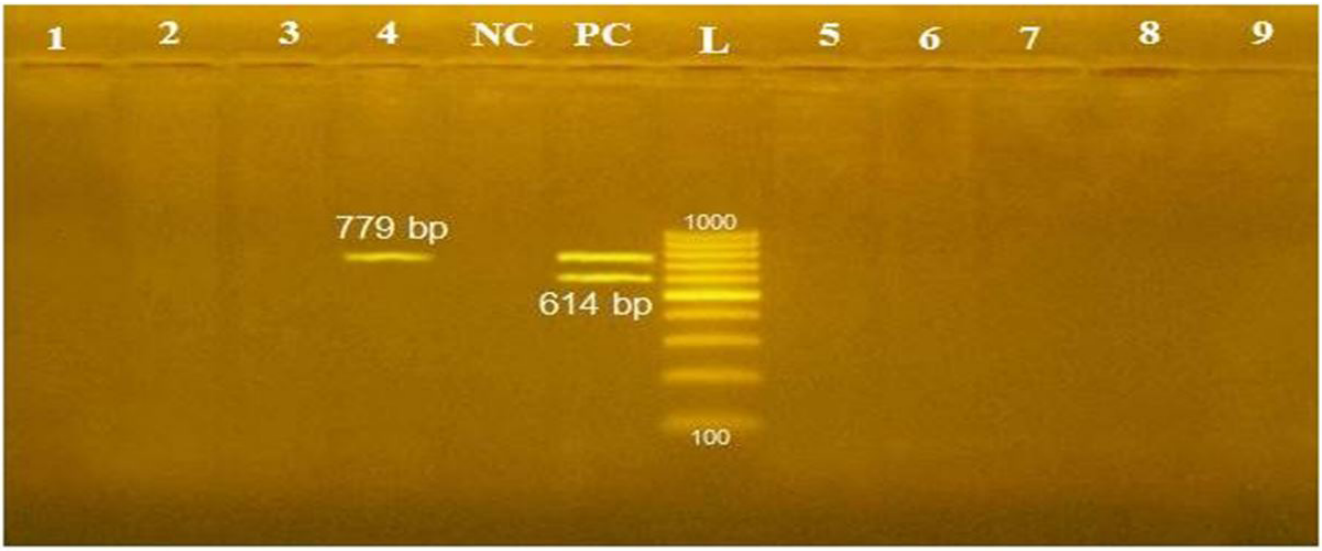
## Molecular detection of virulence genes and ESBL encoding genes

According to the results of multiplex PCR assay, the *Stx1* and *eaeA* were negative in all the isolates (Figure 1 and 2). Only one isolate O112 carried the *Stx2* gene. ESBL-producing *E. coli* isolates carried multiple types of ESBL encoding genes (*blaTEM*) were detected in 100% of isolates, followed by (*blaSHV*) gene were present in 22% (Figure 3,4 and 5). Then (*blaOXA*) gene was present in 11% Table 3 [17,18].

| O Groups | Tested antibiotics | | | | | | | | | | | | Total resistant |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CTX | E | SXT | AK | VA | CN | P | NA | C | TE | NOR | AMC | |
| O125 | R | R | I | S | R | S | R | R | R | R | R | I | 8(66.7%) |
| O29 | R | R | R | S | R | S | R | R | S | R | R | S | 8(66.7%) |
| O157 | I | R | S | S | R | S | R | R | I | R | S | I | 5(41.7%) |
| O86 | S | R | S | S | R | S | R | I | S | R | S | I | 4(33.3%) |
| O148 | R | R | R | S | R | S | R | S | S | R | S | R | 7(58.3%) |
| O127 | R | R | R | S | R | S | R | S | R | R | S | R | 8(66.7%) |
| O27 | S | R | S | S | R | S | R | S | S | R | S | S | 4(33.3%) |
| O112 | I | R | I | S | R | S | R | S | R | R | S | I | 5(41.7%) |
| O20 | S | R | S | S | R | S | R | R | R | R | S | I | 6 (50%) |
| Number of isolates% | 4 (44.4%) | 9 (100%) | 3 (33.3%) | 0 (0%) | 9 (100%) | 0 (0%) | 9 (100%) | 4 (44.4%) | 4 (44.4%) | 9 (100%) | 2 (22.2%) | 2 (22.2%) | |

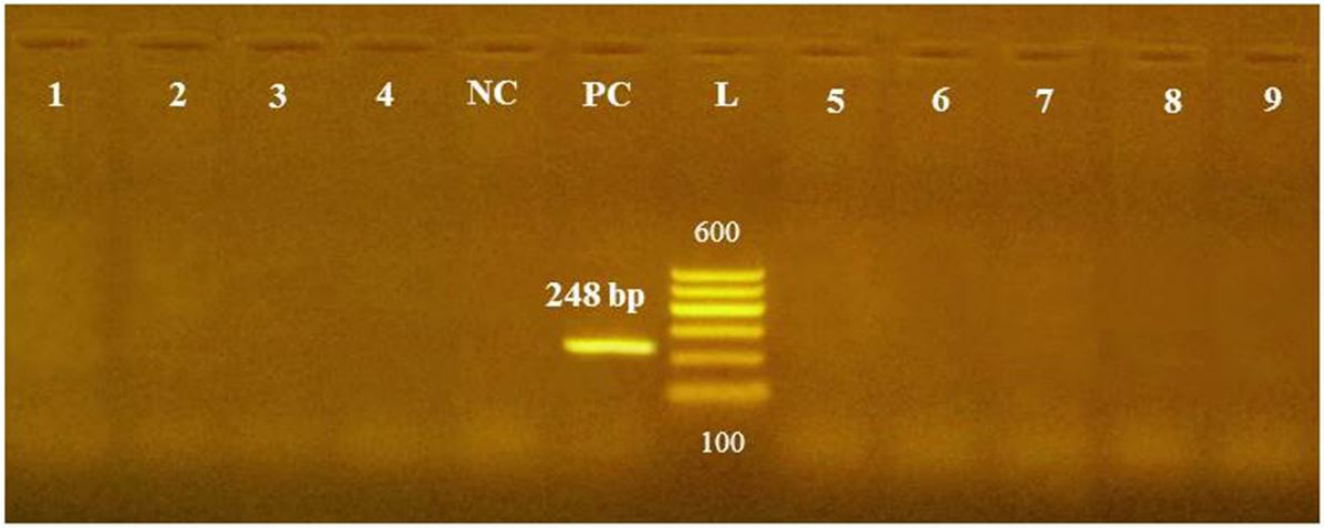
**S**: Sensitive; **R**: Resistant; **I**: Intermediate; **AK**: amikacin; **AMC**: amoxicillin-clavulanic acid; **P:** pencillin; **CTX:** cefotaxime; **NA:** nalidixic acid; **SXT:** sulfamethoxazole-trimethoprim; **TE:** tetracycline; **E:** Erythromycin; **VA:** Vacomycin; **C:** Chloramphenicol; **NOR:** Norfloxacin; **CN:** Gentamycin.

**Table 3:** The results of antibiotic sensitivity test of *E. coli* serogroups isolated from chickens



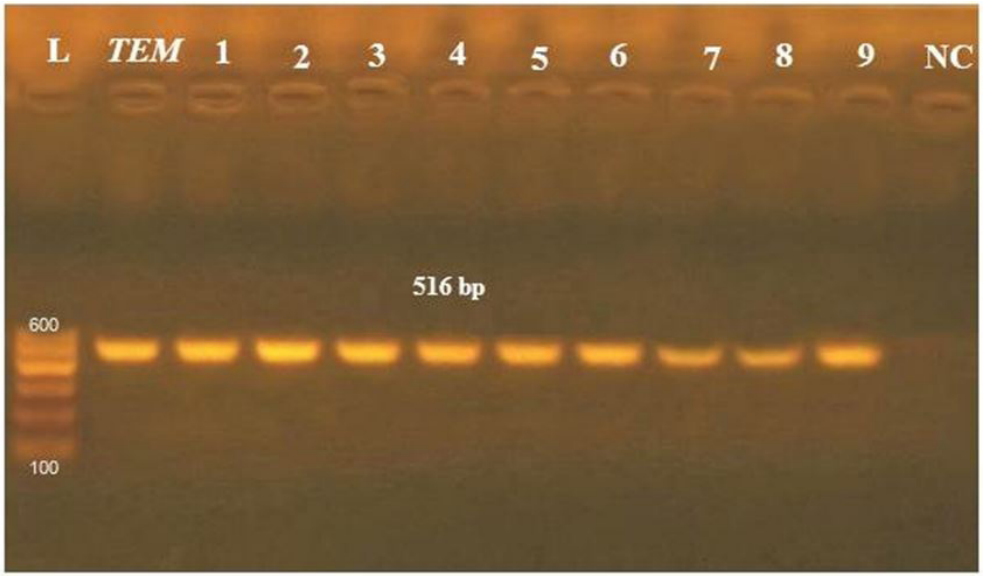

**Lane (L)**: 100 bp DNA ladder; **Lane (PC):** positive control; Lane (NC): negative control; **Lanes (1-9)**: were negative samples for *Stx1*gene; **Lane (4) O112**: positive for *Stx2* gene; **Lanes (1-6, 8-9):** were negative for *Stx2*gene

**Figure 1**: Agar gel electrophoresis showing amplification of 614 and 779 bp product of *Stx1*, *Stx2* of *E. coli* isolated from chickens

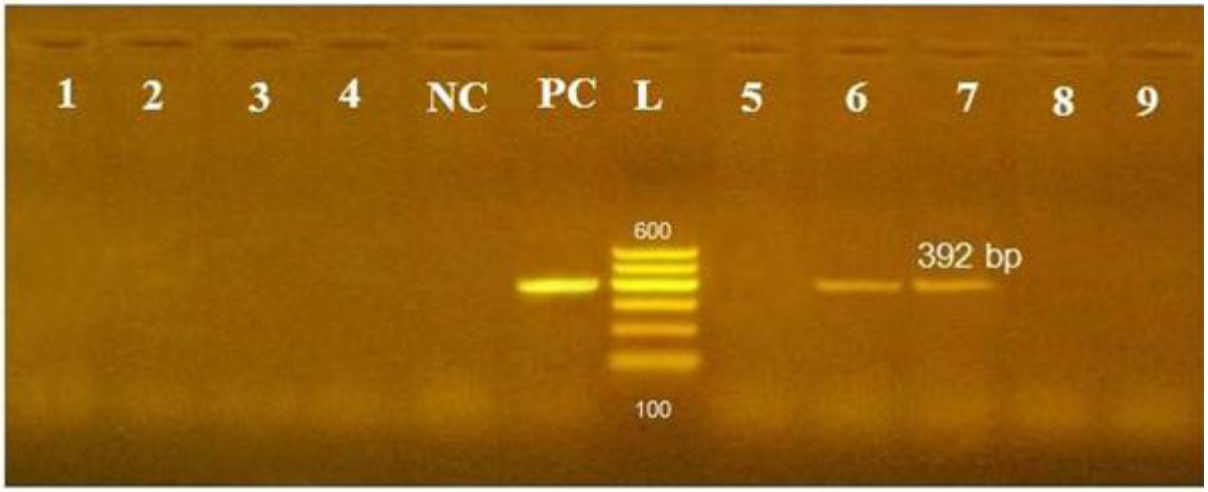



**Lane (L):** 100 bp DNA ladder; **Lane (PC):** positive control; **Lane (NC):** negative control; **Lanes (1-9):** were negative for *eaeA* (intimin) gene

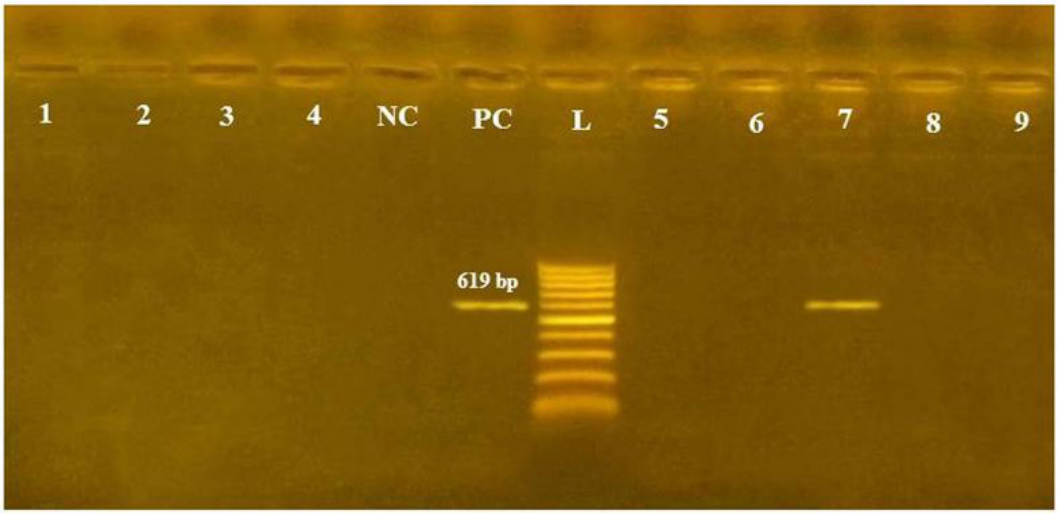
**Figure 2:** Results of PCR for amplification of *eaeA* gene showing amplification of 248 bp product of *E.coli* serogroups isolated from chickens




**Lane (L):** 100 bp DNA ladder; **Lane (PC):** positive control; **Lane (NC):** negative control; **Lanes (1-9):** all isolates were positive for *blaTEM* gene
**Figure 3:** PCR analysis for detection of *blaTEM* gene showing amplification of 516 bp product of *E. coli* isolated fromchickens.




**Lane (L):** 100 bp DNA ladder; **Lane (PC):** positive control; **Lane (NC):** negative control;**Lane (6-7) O127 and O125:** positive for *blaSHV* gene; **Lane (1-5, .8-9.)** were negative for *blaSHV* gene.
**Figure 4:** Agar gel electrophoresis showing amplification of 392 bp product for *blaSHV* gene of *E. coli* isolated from chickens




**Lane (L):** 100 bp DNA ladder; **Lane (PC):** positive control; **Lane (NC):** negative control; **Lane (7) O125:** positive for *blaOXA* gene; **Lane (1-6, 8-9)** were negative for *blaOXA* gene
**Figure 5:** Results of PCR for amplification of *blaOXA* gene showing amplification of 619 bp product of *E. coli* isolated from chickens

## Results

Avian pathogenic *E. coli* (APEC) is the etiological agent of colibacillosis in poultry production [19]. A total of 344 organ samples out of which112 (32.5%) were *E. coli* positive these results mostly agreed with those obtained by Momtaz and Jamshidi were detected that (146/422) 34.59% *E. coli* positive [20]. On the other hand, disagreed with Elsayed, *et al*. detected (166/200) 83% positive [21]. The percentages of *E. coli* isolated from different organs were as the following: Liver (44.1%), Heart (30.2%), Spleen (29%) and Lung (26.7%). This results similar to with other studies in Egypt as those of Roshdy, *et al.* [22] the liver (36.2%) in living diseased, heart (14.3%), and finally the lung (11.9%), those samples were obtained by Abd El Tawab, *et al.* [23] originated from different organs including; Liver (38.2%), Spleen ( 17.6%), heart (16.6%), lung (7.7%) and disagreed with Abd El Tawab, *et al.* [24] distributed as (64%) lung, (51%) liver, (48%) heart blood and (41%) spleen. The serotyping of *E. coli* strains isolated from different organs of chickens (78%) belonged to (9) different 'O' groups, while (22%) strains were untypeable by available antisera. The most commonly detected *E. coli* serogroups isolated from different organs of chickens were O125 (28%), then O127 (12%), O27 (8%), O29 (8%), O86 (7.5%), O112 (7.5%), O148 (7.5%), O20 (2%) and O157 (2%). These results go hand to hand with those recorded by Roshdy, *et al.* [22] O125 (5.5%), O127 (3.2%), O86 (1.8%), O157 (.3%), O148 (.6%) in diseased chickens from different organs, and Abd El Tawab, *et al.* [23] found O125 (15.6%), O27 (3.1%), O20 (3.1%). On the other hand, these results differ from who's reported other serotypes as Elsayed, *et al.* O115 (19.9%), O142 (9.6%), O128 (9.6%), O158 (9.6%), O111 (5.4%), O44 (5.4%), O55 (5.4%), and O157 (15%), O29 (19.9%) [21]. Multidrug resistance was detected in all isolated serotypes (100%) of resistance was observed with Pencillins, Tetracycline, Vancomycin, and Erythromycin antibiotics followed by Chloramphenicol, Cefotaxime and Nalidixic acid were resistance (50%), Sulphamethoxazole / Trimethoprim (40%), Norfloxacin (30%), Amoxicillin/ clavulanic acid (20%), and Amikacin and Gentamycin 0% to all isolates, (Table 2). This results are in line with those recorded by Moawad, *et al.* [25] Penicillin 98.2%, Erythromycin 96.4%, Amoxicillin/clavulanic acid 26.8%, Trimethoprim/sulfamethoxazole 64.3%, Ceftazidime 41.1% and Abbassi *et al.* [26] showed highest rates of resistance observed for tetracycline 74.7%, trimethoprim /sulfamethoxazole, and amoxicillin (each 57%), nalidixic acid 54.4% and ciprofloxacin 34.2%. gentamicin 5.1% this result differs with that obtained from Abd El Tawab, *et al.* [27] which found *E. coli* were highly sensitive to norfloxacin 60%, gentamycin 50%, neomycin 50%, streptomycin 50%. and chloramphenicol 50% but they were moderately sensitive to doxycyclin 10% and erythromycin 40% and highly resistant 100% for amoxicillin /clavulanic acid. According to the results produced by the multiplex PCR assay, the *Stx1* and *eaeA* were negative in all the isolates. Only one isolate O112 carried the *Stx2* gene. ESBL-producing *E. coli* isolates carried multiple types of ESBL encoding genes (*blaTEM*) were detected in 100% of isolates, followed by (*blaSHV*) gene were present in 22%. Then (*blaOXA*) gene was present in 11%, Table 3. These results agree with Oh, *et al.* [28] observed that the Stx genes where negative in all their isolates (n=30) genes. And differ from Byomi, *et al.* [29] found *Stx1* (15.5%), *Stx2* (57.7%). The *eaeA* gene (intimin) similar to Younis, *et al.* [30] detected one strain O26 carried *eaeA* gene. The results also disagreed with Byomi, *et al.* [29] found *eae A* gene present at percent (46.2%) in the isolates. In the case of ESBL genes, (*blaTEM*) was detected in 100% of isolates; followed by (*blaSHV*) gene was present in 22%. Then (*blaOXA*) gene was present in 11%. This result agreed with Abd Tawab, *et al.* [23] that found 100% carry (*blaTEM*) gene, and disagreed with Bardoň *et al.* [31] found 26%. The (*blaSHV*) gene is similar to Rahman, *et al.* [10] detected 20% of isolates carry *blaSHV* gene, disagree with Shehata, *et al.* [28] found 60% carry this gene. The (*blaOXA*) gene, similar with Rahman, *et al.* [10] 20% carry *blaOXA*, in contrast, Shehata, *et al.* [32] was reported 40% carry this gene.

Phenotypic multi-resistance of *E. coli* isolates to β-lactams could be related to the presence of β-lactames encoding genes five isolates (55%) showed a relationship between phenotype and genotype, while four isolates (44%) showed irregular relation, Table 4 and 5 This result agreed with Reich, *et al.* [33] found clinical resistance to cefotaxime was more prevalent in ESBL producers; rate of ceftazidime resistance was higher in SHV-containing isolates.

| Serotypes | PCR results | | | | | |
|---|---|---|---|---|---|---|
| | *Stx1* | *Stx2* | *eaeA* | *bla*TEM | *bla*SHV | *bla*OXA |
| O86 | - | - | - | + | - | - |
| O125 | - | - | - | + | + | + |
| O127 | - | - | - | + | + | - |
| O157 | - | - | - | + | - | - |
| O27 | - | - | - | + | - | - |
| O112 | - | + | - | + | - | - |
| O29 | - | - | - | + | - | - |
| O148 | - | - | - | + | - | - |
| O20 | - | - | - | + | - | - |

**Table 4:** The molecular detection of *E. coli* isolates showing the virulence and β -lactam genes

| Serotypes | Phenotype | | | | Genotype | | | |
|---|---|---|---|---|---|---|---|---|
| | P | CTX | AMC | Resistance No. | *bla TEM* | *bla SHV* | *bla OXA* | Number of gens |
| **O29** | R | R | S | 2 | + | - | - | 1 |
| **O125** | R | R | I | 2 | + | + | + | 3 |
| **O127** | R | R | R | 3 | + | + | - | 2 |
| **O86** | R | S | I | 1 | + | - | - | 1 |
| **O148** | R | R | R | 3 | + | - | - | 1 |
| **O157** | R | I | I | 1 | + | - | - | 1 |
| **O27** | R | S | S | 1 | + | - | - | 1 |
| **O112** | R | I | I | 1 | + | - | - | 1 |
| **O20** | R | S | I | 1 | + | - | - | 1 |
| **Resistance %** | 100% | 44.4% | 22.2% | | 100% | 22.2% | 11.1% | |

**S:** Sensitive; **R:** Resistant; **I:** Intermediate; **AMC:** amoxicillin-clavulanic acid; **P:** pencillin; **CTX:** cefotaxime
**Table 5:** The relationship between phenotype and genotypic β-lactamase resistance *E. coli*

## Conclusion

The present study concluded that chickens represent an important reservoir of multidrug-resistant genes where most administration of these antimicrobials is unnecessary and misuse not excluded, also showed widespread occurrence of ESBL encoding genes which could spread into the food chain. The *blaTEM* gene was the predominant in chickens followed by *blaSHV* gene then *blaOXA* gene in Aswan governorate, Egypt.

## Authors Contributions

DN collected the samples, performing the tests and drafts the manuscript. SS, MW and MA data analysis guided and monitored the entire research work. All authors, read, revised and approved the final manuscript.

## Competing Interests

The authors declare that they have no competing interest.

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