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The Prevalence and Antimicrobial Resistance of Foodborne Bacteria Isolated from Chicken Meat in Local Markets

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Abstract

FOODBORNE pathogens in poultry products represent a major public health concern, particularly with the increasing prevalence of antimicrobial resistance. This study aimed to assess the occurrence, antimicrobial resistance profiles, and resistance genes of *Escherichia coli*, *Salmonella spp.*, and *Staphylococcus aureus* isolated from chicken meat and market environments in El-Fayoum, Egypt. A total of 120 samples were collected and examined bacteriologically, followed by antimicrobial susceptibility testing and PCR detection of selected resistance genes.

The findings revealed prevalence rates of 57.3% for *E. coli*, 5.8% for *Salmonella spp.*, and 29.2% for *S. aureus*. All isolates exhibited multidrug resistance, with *E. coli* showing high resistance to aminoglycosides, β-lactams, tetracyclines, and fluoroquinolones. *Salmonella spp.* and *S. aureus* also demonstrated resistance to most of the tested antibiotics. Molecular analysis identified *bla*CTX-M in 83.3% of *E. coli* isolates, *mec*A in 50%, and *van*A in 33.3% of *S. aureus*, confirming the circulation of ESβL-producing *E. coli*, methicillin-resistant *S. aureus* (MRSA), and vancomycin-resistant *S. aureus* (VRSA).

The sequencing results confirmed the presence of five *E. coli* strains that carry the *bla*CTX-M-14 and *bla*CTX-M-15 genes, as well as one *S. aureus* strain that harbors the *mec*A gene. Phylogenetic tree analysis of the sequenced *E. coli* revealed a high degree of genetic relatedness among them.

The result in this work emphasizes the urgent need for stricter regulations on antimicrobial use in poultry production, improved hygienic practices in local markets, and continuous surveillance to mitigate the spread of resistant pathogens and safeguard consumer health.

Keywords: foodborne bacteria, microbial resistance, resistance genes, food security, gene sequencing.

Introduction

Salmonella species, *Staphylococcus aureus*, and *Escherichia coli* are some of the most worrisome foodborne pathogens and are major causes of morbidity and mortality in poultry [1, 2]. These microorganisms are commonly isolated from poultry and poultry products, where they can thrive in unsanitary environments [3, 4, 5]

Foodborne diseases caused by pathogenic bacteria remain prevalent in both developed and developing countries, the rising consumption of poultry meat is attributed to its nutritional value, but undercooked poultry in fast food establishments serves as a dangerous vehicle for pathogen transmission [6]

E. coli is a member of the *Enterobacteriaceae* family and usually lives harmlessly in the intestines of human and animals, but it can cause infections outside of the intestines as well. Extra-intestinal pathogenic *E. coli* (ExPEC) is a genetically diverse group of bacteria that can live in many different environments. Enterohemorrhagic strains produce poisons that are similar to those of Shigella dysenteriae, which causes gastroenteritis [7].

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Salmonella enterica, especially *Salmonella typhimurium* and *Salmonella enteritidis*, are the most important types of *Salmonella* that make people sick. Chicken products are frequently infected with *Salmonella*, which can be dangerous for anyone who touches them or consumes undercooked meat so that foodborne salmonellosis is still common [8].

Healthy individuals and animals typically have *Staphylococcus aureus* in their skin and nasal passages without any problems. Some types, called enterotoxigenic bacteria, it can produce enterotoxins that can induce Staphylococcal food poisoning (SFP) when eaten in contaminated food [9].

To safeguard public health and assure that food is safe, it is very important to understand how resistance works and the genetic elements that let these diseases survive in the food chain [10]. Antimicrobial resistance (AMR) poses a serious threat to public health around the world [11]. Among the many causes of antimicrobial resistance, the antibiotics that are frequently used as growth boosters in chicken production in addition to their therapeutic uses, the misuse of antibiotics in agriculture at all, and the improper food handling procedures may result in the development of resistant bacterial strains. This occurrence emphasises the need for thorough monitoring and investigation into the resistance patterns of common infections [12]. Aminoglycosides, cephalosporins, tetracyclines, and fluoroquinolones are among the panel of antibiotics that should be used in a thorough antimicrobial sensitivity test since chicken industry often uses these antibiotics; their inclusion in these tests enables a comprehensive evaluation of antibiotic resistance in the isolated strains [13, 14].

Extended-spectrum beta-lactamases (ESβLs), particularly those produced by the *bla*CTX-M gene, pose a substantial public health risk because they confer antibiotic resistance to bacteria such as *E. coli* and *Salmonella*, making infections more difficult to cure [15]. ESβLs are enzymes that degrade beta-lactam antibiotics, such as penicillins and cephalosporins, leaving them useless against afflicted bacteria. Understanding the incidence of ESβLs, particularly in poultry, is critical for determining the danger of these resistant bacteria transmitting to humans through the food chain or other interactions [16, 17]. Equally concerning, and given the difficulties in treating, the infections caused by methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA), their presence in poultry habitats raises major public health problems [18]. MRSA acquires resistance through the *Staphmec*A gene, which codes for a mutated penicillin-binding protein (PBP2a) with low affinity for beta-lactam antibiotics [19]. Conversely, VRSA is resistant to vancomycin and other glycopeptide antibiotics by use of the *Staphvan*A gene, which alters cell wall peptidoglycan [20].

Examine the genetic foundation of resistance by employing polymerase chain reaction (PCR) methods to amplify certain resistance genes, verify their existence and offer insights into their distribution across various strains [21]. Sequencing of the isolates allows exploring the genetics behind antibiotic resistance, this approach helps to identify resistance genes, and track how they evolve, and reveal connections between different isolates [22].

The main goals of this study were to investigate the prevalence of harmful bacteria from chicken meat that could cause foodborne illnesses such as *E. coli*, *Salmonella* species, and *S. aureus*, and to analyze their antibiotic resistance profiles. This involves determining the prevalence of some resistance genes, including *bla*CTX-M, *mec*A, and *van*A, investigate the genetic connections among the isolates and comprehend the mechanisms of resistance by using PCR and sequencing methodologies.

Material and Methods

Samples

The survey was conducted in the El-Fayoum Province of Egypt. 120 samples of chicken thigh, liver, and chest (forty samples from each) and thirty environmental swabs from walls and utensils (rotated and rubbed several times) were collected from commercial chicken stores and marketplaces in various provinces during the winter season, between November and December 2025. Within two hours of collection, samples were transported to the lab in an icebox using sterile plastic bags [23].

Isolation of bacterial pathogens and their biochemical identification: conducted according to Quinn *et al*. [24].

For *E. coli* 1 g of each meat sample, in addition to the collected environmental swabs, were suspended in Tryptone Soya broth and incubated at 37°C for the entire night, a loopful of the broth was streaked over MacConkey agar and incubated aerobically at 37°C for 24 hours, *E. coli* (pink colony; lactose fermenter) was examined on the plates. For metallic sheen, one isolated colony was subcultured on Eosin Methylene Blue (EMB) agar.

For *salmonella* isolation, 1 g of the sample pre-enriched with 0.1% w/v buffered peptone water (Oxoid) and incubated for 24 hours at 37°C. 1 mL of pre-enriched buffered peptone was added to 10 mL of selective enrichment media, Rappaport Vassiliadis Soya bean broth (RVS) (Oxoid), The mixture was then incubated for 24 hours at 41°C. After that one loopful of RVS was being moved to S-S agar, incubated for 24 to 48 hours at 37°C. *Salmonella* colonies on S-S agar plates were colorless or translucent and had black centers. *Salmonella* and *E. coli* were confirmed by biochemical tests such as Simmon's Citrate, Methyl Red-Voges Proskauer (MR-VP), and Indole Production.

Staphylococci: Baird Parker agar (Oxoid) was used for the initial isolation of *Staphylococci*, and it is incubated at 37°C for the entire night. On Baird-Parker agar, *S aureus* colony are typically black or dark gray, shiny, and convex, measuring 1-2.5 mm. A clear zone around the colony indicates proteolysis or lipolysis from egg yolk breakdown. An opaque ring within this zone suggests Lecithinase activity. Other *Staphylococci* or bacteria that lack these enzymes will not form these zones, aiding in the differentiation of *S. aureus*

Single recovered colonies were cultivated for the entire night at 37°C on 5% citrated sheep blood agar plates. Biochemical testing typically identifies *S. aureus* as coagulase-positive while *S. epidermidis* is not. In contrast to *S. epidermidis*, *S. aureus* ferments mannitol and, after 24 hours, forms yellow colonies on mannitol salt agar at 37°C.

Antimicrobial susceptibility test:

Isolates of *E. coli* (N=27), *Salmonella spp*. (N=6), and *S. aureus* (N=15) were examined against several antibiotic discs (Oxoid), purified colonies were cultured in Muller Hinton broth overnight; the inoculum optical density was then adjusted to 0.5 McFarland Standard, and 100 µl was spread out on the Muller Hinton agar, and the surface of the inoculated plates was loaded with antibiotic discs with their respective concentrations as mentioned in Tables (5, 6 and7). Following incubation for 24 h at 37°C, each antibiotic's inhibition zones were determined and recorded and then interpreted according to the Clinical and Laboratory Standards Institute (CLSI) standard 2021 [25].

Molecular characterization

DNA extraction from samples was performed using the QIAamp DNA Mini kit with modifications. Briefly, the sample suspension was incubated with proteinase K and lysis buffer. After incubation, ethanol was added to the lysate. The sample was then washed and centrifuged. Nucleic acid was eluted with elution buffer. Primers used, supplied from Metabion, with primer sequences, amplified segment, primary denaturation, amplification cycles, and final extension with references, are listed in table (1). Primers were utilized in a reaction containing Emerald Amp Max PCR Master Mix, primers, water, and DNA template. The reaction was performed in a thermal cycler. The products of PCR were separated by electrophoresis on agarose gel in TBE buffer. For gel analysis, PCR products were loaded in each gel slot. The Gene Ruler 100 bp ladder was used to determine the fragment sizes. The gel was photographed by a gel documentation system, and the data was analyzed using computer software.

Sequencing

PCR products were purified using the QIA quick PCR Product extraction kit. (Qiagen, Valencia). Big Dye Terminator V3.1 Cycle Sequencing Kit (Perkin-Elmer) was used for the sequence reaction, and then it was purified using a Centrisep spin column. DNA sequences were obtained by an Applied Biosystems 3130 genetic analyzer (HITACHI, Japan), and a BLAST® analysis (Basic Local Alignment Search Tool) was initially performed to establish sequence identity to GenBank accessions [29]. The phylogenetic tree was created by the Meg Align module of Laser gene DNA Star version 12.1 [30], and phylogenetic analyses were done using maximum likelihood, neighbor joining, and maximum parsimony in MEGA7 [31].

Results

Isolation and identification of bacterial pathogens

From a total of 120 samples of various chicken meat parts, 86 isolates (57.3%) were confirmed through bacteriological analysis to be *E. coli.* This included 21 isolates from liver, 19 from chest, and 23 from thigh. Additionally, 23 *E. coli* isolates were recovered from 30 environmental swabs (Table 2, Fig. 1).

In the attempt to isolate *Salmonella spp.* from the same chicken meat samples, a total of 7 isolates (5.8%) were confirmed positive. These included three from the liver, two from the chest, and two from the thigh (Table3, Fig.1).

Out of the 120 chicken meat samples collected, a total of 79 isolates of *Staphylococcus spp.* were identified, representing 56.8%. These included 26 isolates from the liver, 22 from the chest, and 31 from the thigh. The *Staphylococcus spp* isolates were subsequently differentiated biochemically into *S. aureus* 35 (29.2) and *S. epidermidis* 44 (36.7) (Table 4, Fig.1).

Antimicrobial resistance:

All 27 *E. coli* isolates that were examined were completely resistant (100%) to streptomycin, gentamicin, cefotaxime, oxytetracycline, amoxicillin, and erythromycin. Resistance rates for chloramphenicol, levofloxacin, colistin sulphonate, and ciprofloxacin were 77.8%, 74.1%, 63%, and 59.35%, respectively. Amikacin had the lowest resistant rate (3.7%), while levofloxacin, chloramphenicol, and colistin sulphonate had intermediate resistance at 7.4%, 11.1%, 14.8%, and 29.6%, respectively (Table 5)

All six of the *Salmonella* isolates that were tested were resistant to eight out of ten of the used antibiotic discs (streptomycin, gentamicin, cefotaxime, colistin sulphonate, ciprofloxacin, levofloxacin, amoxicillin, and chloramphenicol), while 66.7% of them were resistant to oxytetracycline. On the other hand, all isolates (100%) were sensitive to amikacin (Table 6).

All 15 *S. aureus* isolates (100%) were resistant to oxytetracycline, oxacillin, flucloxacillin, erythromycin, vancomycin, and chloramphenicol. While gentamicin and neomycin were only 60% resistant. The lowest resistance level found was 46.7% for both cefepime and ciprofloxacin. Gentamicin had an intermediate resistance rate of 6.7%, and ciprofloxacin had an intermediate resistance rate of 20% (Table 7).

Molecular genetic analysis:

In the present study, the β-lactamase-related gene *bla*CTX-M was detected in 5/6 of the *E. coli* isolates tested (N=6) with a frequency of 83.33%. As for *Salmonella spp*., none of the tested isolates (N=3) showed the targeted gene (0%) (Table 8, Fig. 2). Among six selected *S. aureus*, three isolates (50%) showed the *mec*A gene and two (33.33%) demonstrated *van*A (Table 9, Fig. 3&4).

Sequencing results:

The sequencing results of the *E. coli bla*CTX-M and *S. aureus mec*A genes offer clues about the genetic diversity of antimicrobial resistance in bacterial strains isolated from chicken meat in Fayoum, Egypt. The data, as summarized in (Table 10), confirmed the presence of five *E. coli* strains carrying the *bla*CTX-M-14 and *bla*CTX-M-15 genes and one *S. aureus* carrying the *mec*A gene.

Phylogenetic tree analysis:

Phylogenetic tree analysis (Fig. 5) carried out for the sequenced five *E. coli bla*CTX-M strains detected in the present study showed the closeness in sequence and ancestor of both PV386790 *bla*CTX-M-14 and PV386791 *bla*CTX-M-14 strains, with bootstrap support of 90%, while the other three strains, PV386789 *bla*CTX-M-15, PV386792 *bla*CTX-M-15 and PV386793 *bla*CTX-M-15, shared a different most recent ancestor and sequence closeness reflected by a bootstrap support of 99%.

Discussion

Poultry meat is a significant food source that is susceptible to contamination by harmful bacteria. The presence of *E. coli*, *Salmonella spp.*, and *S. aureus* in chicken meat poses considerable public health risks, raising concerns about foodborne illnesses associated with poultry consumption, particularly in regions with varying standards of food safety. These bacteria can cause symptoms such as diarrhea, vomiting, abdominal cramps, and fevers [32]. Infections caused by *Salmonella* can be particularly severe, potentially resulting in hospitalization and even death [33]. *S. aureus* produces heat-stable toxins that can lead to food poisoning even after the meat has been cooked [34]. Previous research has highlighted significant contamination levels in poultry meat, which emphasises the value of thoroughly evaluating microbiological risks within the market.

By examining samples from various parts of chicken, including the liver, breast, and thigh, this study aims to provide a more comprehensive understanding of the prevalence of contamination in chicken meat. The detection of 86 isolates of *E. coli* (57.3%) from 120 chicken meat samples indicates a significant level of contamination. The findings reveal a considerable level of contamination, underscoring the necessity for stringent food safety measures.

The distribution of *E. coli* isolates across different chicken parts (Table 2) suggests that certain meat parts may be more disposed to bacterial contamination. The recovery of 23 *E. coli* isolates from 30 environmental swabs further emphasises the likely role of the surrounding environment in the transmission of these pathogens. Hygienic weak points during meat processing are significant sources of chicken meat contamination. Bacteria found in the bird's gastrointestinal tract can contaminate the carcass during slaughtering and evisceration. The environment of the slaughterhouse, which includes surfaces, air, and water, can also be a source of contamination. Additionally, processing equipment can harbor and disseminate bacteria, while workers' hands may directly contaminate meat [35], thus necessitating improved hygiene practices in poultry processing and handling.

The prevalence of *E. coli* in this study falls within the wide range of prevalence rates reported in many other studies in Egypt and worldwide, indicating that *E. coli* contamination in chicken meat is a common issue. A study in Minia, Egypt, found *E. coli* in 73% of poultry meat and products [36]. A study in Romania found a 30% prevalence [37], another in El Salvador reported a 74% prevalence [38]. And another study in Jember, Indonesia, found that 100% of chicken meat samples were contaminated with *E. coli* [39].

The isolation of only 7 *Salmonella spp*. (5.8%) indicates a relatively lower prevalence compared to *E. coli*. However, the serious health consequences associated with *Salmonella* infections should be considered. Study two main poultry abattoirs in Lusaka, Zambia. *Salmonella* contamination detected in 2.5% of the selected dressed chickens [40], while research in El Salvador reported *Salmonella spp*. in only 1% of chicken meat [38]. A study in Zagazig city, Egypt, found *Salmonella spp.* in 6.66% of chicken meat products [41]. A higher isolation rate (35%) of *Salmonella* from poultry meat and products in Minia, Egypt was also recorded [36].

The identification of 79 *Staphylococcus spp*. isolates (56.8%) strengthen the concern regarding bacterial contamination in poultry products. The differentiation between *S. aureus* (35 isolates) and *S.*

epidermidis (44 isolates) is particularly remarkable. *S aureus*, known for its pathogenic potential and ability to produce enterotoxins, poses a significant risk for foodborne illnesses. The presence of a high percentage of *S. epidermidis*, even if it is generally less pathogenic, indicates overall contamination levels. A review of studies in African countries found that a little over 20% of chicken meat samples were contaminated with *S. aureus* [42]. Another study found the occurrence of *S. aureus* in raw chicken meat was 38.82% [43]. Also, a recent study by Hamad *et al*. recorded *S. aureus* in breast and thigh samples at 92% and 84%, respectively [44]. in Egypt, Morshdy *et al* found *S. aureus* in 22% of chicken meat products [41], while El-Sayed *et al.*, found *S. aureus* in, 32% of fresh chicken thigh and 32% of fresh chicken breast [45].

Variations in bacterial isolation rates in chicken meat stem from differences in hygiene practices at the farm, processing plant, and retail levels. Processing methods such as scalding, evisceration, and chilling can either reduce or promote cross-contamination depending on the effectiveness of temperature control, equipment sanitation, and process design. Geographical location influences contamination through environmental factors like climate and water sources, as well as regional farming and processing practices, ultimately affecting the prevalence and types of bacteria found in chicken meat [35, 46].

Multiple studies confirm that inadequate hygiene during slaughtering, processing, and handling significantly contributes to increasing the risk of bacterial contamination of chicken meat with various bacteria, including *E. coli, S. aureus, and Salmonella spp.* [38, 40, 46].

The present study reveals alarming rates of antimicrobial resistance (AMR) among bacterial isolates from chicken meat, raising significant public health concerns. The overuse of antibiotics in poultry production contributes to the selection and spread of resistant bacteria, which can then be transmitted to humans through the food chain or direct contact. [12, 47]

The findings that all 27 *E. coli* isolates tested were MDR and completely resistant (100%) to streptomycin, gentamicin, cefotaxime, oxytetracycline, amoxicillin, and erythromycin is particularly concerning, which can complicate treatment options for infections. Chloramphenicol, levofloxacin, colistin sulphonate, and ciprofloxacin showed lower resistance rates (77.8%, 74.1%, 63%, and 59.35%, respectively). The low resistance to amikacin (3.7%) may suggest its continued effectiveness against *E. coli* in this situation, but continuous monitoring is essential to prevent the emergence of resistance.

The *Salmonella* isolates displayed complete resistance to eight out of the ten antibiotics tested (streptomycin, gentamicin, cefotaxime, colistin sulphonate, ciprofloxacin, levofloxacin, amoxicillin, and chloramphenicol). The resistance to oxytetracycline was 66.7%. The high resistance levels observed in *Salmonella spp*. are consistent with global trends, where *Salmonella* isolates from poultry often exhibit resistance to multiple antimicrobials. The fact that all isolates were sensitive to amikacin is a positive finding, similar to *E. coli*.

All the 15 *S. aureus* isolates (100%) were resistant to oxytetracycline, oxacillin, flucloxacillin, erythromycin, vancomycin, and chloramphenicol. This is a critical finding, especially the 100% resistance to oxacillin and flucloxacillin, which are related to methicillin resistance, indicating the presence of methicillin-resistant *Staphylococcus aureus* (MRSA). The high resistance to vancomycin, a last-resort antibiotic, is extremely worrisome. The lower resistance levels to gentamicin and neomycin (60%) and cefepime and ciprofloxacin (46.7%) might offer some therapeutic alternatives, but the overall resistance profile of *S. aureus* is alarming.

Numerous studies have documented the presence of MDR *E. coli, Salmonella spp.*, and *S. aureus* in chicken meat across various regions and pointed out the possible risks associated with chicken consumption. Research in Bangladesh revealed that *E. coli* and *S. aureus* isolates from chicken meat were 100% resistant to amoxicillin and erythromycin, where all isolates being multidrug-resistant [48]. A study in Jember, Indonesia, found that all of the isolated *E. coli* from chicken meat samples was multidrug-resistant and showed 100% resistance to cotrimoxazole and cefixime [39]. A study in East Java, Indonesia, found that 42% and 43% of *E. coli* and *Salmonella* spp. isolated from chicken meat were multidrug-resistant, with high resistance observed against amoxicillin, ampicillin, and oxytetracycline [49]. A study in Bangladesh found that all MRSA isolates from chicken meat were multidrug-resistant where the highest rates of resistance were observed against cefoxitin (100%), followed by nalidixic acid, ampicillin, and oxacillin [50]. Researches conducted in Egypt also showed that isolates of *Salmonella* and *E. coli* from chicken meat demonstrated strong resistance to ampicillin, trimethoprim-sulfonamide, and metronidazole [36]. Additionally, MDR has been observed in *S. aureus* isolates from chicken meat products [41].

To combat antibiotic resistance in poultry, responsible antibiotic use with accurate diagnoses and prescriptions and reduced antibiotic use through improved biosecurity, hygiene and vaccination programs should be followed [51]. Alternative strategies may also be included, such as probiotics, prebiotics, phytogenics, bacteriophages, and

antimicrobial peptides, to improve gut health and boost immunity [52].

The PCR results indicating the presence of specific resistance genes (*bla*CTX-M, *mec*A, and *van*A) in *E. coli and S. aureus,* isolates provide a genetic basis for the observed antimicrobial resistance phenotypes.

The detection of the *bla*CTX-M gene in 83.33% of *E. coli* isolates signifies a high prevalence of extended-spectrum beta-lactamase (ESβL)-producing *E. coli.* The *bla*CTX-M gene encodes for enzymes that confer resistance to extended-spectrum cephalosporins like cefotaxime, ceftriaxone, and ceftazidime. Many studies have shown a strong correlation between the presence of *bla*CTX-M and extended-spectrum cephalosporins resistance in *E. coli* [53, 54]. The *bla*CTX-M genes are often located on mobile genetic elements such as plasmids, facilitating their horizontal transfer between bacteria; this can lead to rapid dissemination of resistance within and across different bacterial species [55].

The absence of the *bla*CTX-M gene in *Salmonella* isolates suggests that ESβL-mediated resistance may be less common in the *Salmonella* population in this study. Other mechanisms, such as efflux pumps or target site mutations, might be responsible for the observed resistance in *Salmonella* isolates in this study [56].

Screening of the methicillin resistance (*mec*A) gene revealed that 43.5% of the tested isolates of *S. aureus* were positive. The high prevalence of MDR-MRSA in chicken meat samples in this study give attention to the need for hygiene instructions among food handlers, focusing on their possible role as reservoirs and transmitters of MRSA [50]. The *mec*A gene encodes a modified penicillin-binding protein (PBP2a) with low affinity for beta-lactam antibiotics, including methicillin, oxacillin, and cephalosporins [57]. A significant correlation exists between the presence of the *mec*A gene and resistance to oxacillin, gentamicin, erythromycin, and clindamycin [58]. Ryffel *et al.* also recorded *mec*A gene in 50% of *S. aureus* isolates [57].

The detection of the *van*A gene in 33.33% of *S. aureus* isolates indicates the presence of vancomycin-resistant *S. aureus* (VRSA) and confers resistance to the glycopeptide antibiotic vancomycin, the last-resort treatment for MRSA infections [59, 60, 61]. The absence of the *van*A gene in some vancomycin-resistant *S. aureus* isolates, despite their resistance phenotype, may arise because *van*A is not the sole mechanism conferring resistance. Alternative mechanisms include cell wall thickening and modifications to the cell wall's building blocks (the excess D-Ala-D-Ala residues), which reduces vancomycin's ability to reach its target [62]. Mutations in regulatory genes can also lead to reduced drug influx or increased efflux. Furthermore, the capacity to form biofilms may also play a role in vancomycin tolerance [63].

The co-existence of MRSA and VRSA in the same
S. aureus isolates from chicken meat represents a significant public health threat. This phenomenon may occur through horizontal gene transfer, whereby MRSA acquires the *van*A gene from vancomycin-resistant *Enterococci* (VRE), often driven by selective pressure resulting from antibiotic overuse. Additionally, biofilms can enhance antibiotic resistance and promote the transfer of resistance genes. [18, 64]. The emergence of this "superbug" is particularly concerning because it poses a direct risk to humans through colonization or infection resulting from handling or consuming contaminated poultry products, and such infections are extremely difficult to treat due to the limited number of effective antibiotics available [64, 65].

The slaughterhouse environment can serve as a critical hotspot for the dissemination of antibiotic-resistant genes, as it often harbors a wide range of bacteria originating from animals, processing equipment, wastewater, and surfaces. Inadequate sanitation, improper waste disposal, and the accumulation of organic matter create favorable conditions for bacterial survival and gene transfer. Additionally, the close interaction of commensal, pathogenic, and environmental bacteria facilitates horizontal gene transfer, thereby enhancing the persistence and spread of resistance genes within the slaughterhouse setting

Next-generation sequencing is expanding our abilities to detect and study antimicrobial resistance [66]. The sequencing in this work (Table 10,) significantly strengthens our findings on antibiotic resistance in bacteria from chickens' meat, *bla*CTX-M-15 and *bla*CTX-M-14: These genes are common in antibiotic-resistant *E. coli* and *mec*A in *S. aureus,* which confirms the presence of MRSA. Finding them in chicken samples in Egypt suggests these strains may be widespread.

Phylogenetic analysis of the *blaCTX-M* genes (Fig. 5) revealed sequence similarity among the *E. coli* strains, suggesting a possible clonal origin or horizontal gene transfer of the resistance genes. The close relationship between the *blaCTX-M-14* strains indicates a recent common ancestor or transmission event. Similarly, the high bootstrap support (99%) for the cluster of *blaCTX-M-15* strains suggests a shared evolutionary history. Similar findings have been reported in other studies, which have shown the dissemination of specific *blaCTX-M* types within certain geographical areas [67, 68]. The presence of closely related ESβL-producing *E. coli* strains in chicken meat suggests a potential risk of transmission and highlights the need for effective

control measures to prevent the spread of antibiotic resistance in the food chain.

Conclusion

The detection of multidrug-resistant *E. coli*, *Salmonella spp.*, and *S. aureus* that harbour critical resistance genes in chicken meat and market environments highlights a serious and growing public health concern. The presence of extended-spectrum β-lactamase (ESβL)-producing *E. coli*, methicillin-resistant *S. aureus* (MRSA), and vancomycin-resistant *S. aureus* (VRSA) in retail poultry products increases the risk of transmission of these pathogens and their resistance determinants to consumers, either directly through foodborne infections or indirectly via the transfer of resistance genes to other bacteria in the human gut. This situation not only complicates treatment options but also contributes to the global burden of antimicrobial resistance. Therefore, strengthening antimicrobial stewardship in poultry farming, enforcing strict hygienic and biosecurity measures throughout the slaughtering and marketing processes and implementing continuous surveillance programs are essential to limit the dissemination of resistant pathogens. Such integrated measures are critical to safeguarding public health and ensuring the sustainability of poultry production systems.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

TABLE 1. Primers sequences, target genes, amplicon sizes and cycling conditions

Target gene	Primers sequences	Amplified segment (bp)	Amplification (35 cycles)				Final extension	Reference
			Primary denaturation	Secondary denaturation	Annealing	Extension		
Staph*mecA*	F: GTA GAA ATG ACT GAA CGT CCG ATA A R:CCA ATT CCA CAT TGT TTC GGT CTA A	310	94˚C 5 min.	94˚C 30 sec.	50˚C 30 sec.	72˚C 30 sec.	72˚C 10 min.	[26]
Staph*vanA*	F:CAT GAC GTA TCG GTA AAA TC R; ACC GGG CAG RGT ATT GAC	885			50˚C 40 sec.	72˚C 50 sec.	72˚C 10 min.	[27]
E. coli and Salmonella *BlaCTX-M*	F: ATG TGC AGY ACC AGT AAR GTK ATG GC R: TGG GTR AAR TAR GTS ACC AGA AYC AGC GG	593			54˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	[28]

TABLE 2. The prevalence rate of *E. coli* among the different tested chicken meat parts and environment.

No. of examined samples		Liver	Chest	Thigh	Environmental swabs	Total
		40	40	40	30	150
Positive isolation for *E. coli*	NO.	21	19	23	23	86
	%	52.2%	47.5%	57.5%	76.67%	57.33%

* The percentage was calculated according to the total number of collected samples from each organ

TABLE 3. The prevalence rate of *Salmonella spp*. from different tested chicken meat parts

No. of examined samples		Liver	Chest	Thigh	Total
		40	40	40	120
Positive isolation for *Salmonella spp.*	NO.	3	2	2	7
	%	7.5%	5%	5%	5.83%

* The percentage was calculated according to the total number of collected samples from each organ

TABLE 4. The prevalence rate of *Salmonella spp*. from different tested chicken meat parts

No. of examined samples		Liver	Chest	Thigh	Total
		40	40	40	120
Positive isolation for *Staphylococcus spp.*	NO.	26	22	31	79
	%	65%	55%	77.5%	56.83%
S. aureus	NO.	13	10	12	35
	%	32.5%	25%	30%	29.16
S. epidermidis	NO.	13	12	19	44
	%	32.5%	30%	47.5%	36.67

* The percentage was calculated according to the total number of collected samples from each organ

TABLE 5. Antimicrobial susceptibility patterns of *E. coli* isolated from chicken meat and environment:

Antimicrobial classes	Antibacterial agents	Disc content (µg/disc)	*Escherichia coli* (N=27)					
			S		IM		R	
			NO	%	NO	%	NO	%
AMINOGLYCOSIDES	Amikacin (Ak)	30	24	88.9%	2	7.4%	1	3.7%
	Streptomycin (S)	10	0	0%	0	0%	27	100%
	Gentamicin (CN)	10	0	0%	0	0%	27	100%
CEPHALOSPORINS Beta-lactams	Cefotaxime (CTX)	10	0	0%	0	0%	27	100%
TETRACYCLINE'S	Oxytetracycline (OT)	30	0	0%	0	0%	27	100%
POLYPEPTIDES	Colistin sulphonate (CL)	10	3	11.1%	8	29.6%	16	59.3%
FLUOROQUINOLONES	Ciprofloxacin (CIP)	5	10	37%	0	0%	17	63%
	Levofloxacin (LE)	5	4	14.8%	3	11.1 %	20	74.1%
PENICILLINS	Amoxicillin (AML)	10	0	0%	0	0%	27	100%
Macrolide	Erythromycin (E)	15	0	0%	0	0%	27	100%
Amphenicol	Chloramphenicol (C)	30	2	7.4%	4	14.8%	21	77.8%

TABLE 6. Antimicrobial susceptibility patterns of *Salmonella spp.* isolated from chicken meat:

Antimicrobial classes	Antibacterial agents	Disc content (µg/disc)	*Salmonella spp*. (N=6)					
			S		IM		R	
			NO	%	NO	%	NO	%
AMINOGLYCOSIDES	Amikacin (Ak)	30	6	100%	0	0%	0	0%
	Streptomycin (S)	10	0	0%	0	0%	6	100%
	Gentamicin (CN)	10	0	0%	0	0%	6	100%
CEPHALOSPORINS Beta-lactams	Cefotaxime (CTX)	10	0	0%	0	0%	6	100%
TETRACYCLINE'S	Oxytetracycline (OT)	30	2	33.3%	0	0%	4	66.7%
POLYPEPTIDES	Colistin sulphonate (CL)	10	0	0%	0	0%	6	100%
FLUOROQUINOLONES	Ciprofloxacin (CIP)	5	0	0%	0	0%	6	100%
	Levofloxacin (LE)	5	0	0%	0	0%	6	100%
PENICILLINS	Amoxicillin (AML)	10	0	0%	0	0%	6	100%
Amphenicol	Chloramphenicol (C)	30	0	0%	0	0%	6	100%

TABLE 7. Antimicrobial susceptibility patterns of *Staphylococcus aureus* isolated from chicken meat:

Antimicrobial class	Antibacterial agents	Disc content (µg/disc)	*Staphylococcus aureus* (N=15)					
			S		IM		R	
			NO	%	NO	%	NO	%
AMINOGLYCOSIDES	Gentamicin (GN)	10	5	33.3%	1	6.7%	9	60%
	Neomycin (N)	30	6	40%	0	0%	9	60%
CEPHALOSPORINS Beta-lactams	Cefepime (CPM)	30	8	53.3%	0	0%	7	46.7%
TETRACYCLINE'S	Oxytetracycline (OT)	30	0	0%	0	0%	15	100%
FLUOROQUINOLONES	Ciprofloxacin (CIP)	5	5	33.3%	3	20%	7	46.7
PENICILLINS	Oxacillin (OXA)	1	0	0%	0	0%	15	100%
	Flucloxacillin (FLU)	5	0	0%	0	0%	15	100%
Macrolide	Erythromycin (E)	15	0	0%	0	0%	15	100%
Glycopeptide	Vancomycin (VA)	30	0	0%	0	0%	15	100%
Amphenicol	Chloramphenicol (C)	30	0	0%	0	0%	15	100%

TABLE 8. prevalence of the *bla*CTX-M gene among the *E. coli* and *Salmonella* isolates

Bacteria	Sample	*BlaCTX-M*
E. coli	1	+
	2	+
	3	+
	4	-
	5	+
	6	+
	Total frequency of the gene	5/6 (83.3%)
Salmonella	7	-
	8	-
	9	-
	Total frequency of the gene	0/3 (0%)

TABLE 9. prevalence of *mec*A and *van*A genes among *S. aureus* isolates

Bacteria	Sample	*mec*A	*van*A
S. aureus	1	+	+
	2	-	-
	3	-	-
	4	+	-
	5	+	+
	6	-	
Total frequency of the gene		3/6 (50%)	2/6 (33.33%)

TABLE 10. Summary of GenBank Submission Data for *E. coli bla*CTX Gene and *S. aureus mec*A Gene

Accession	Strain	Gene	Collection Date	Isolation Source	Host	Translation (First 20 Amino Acids)
PV386789	*E. coli*	*bla*CTX-M-15	Jan-2025	Faium Egypt	Thigh-muscle	MAAAAVLKKSESEPNLLNQR
PV386790	*E. coli*	*bla*CTX-M-14	Jan-2025	Faium Egypt	Thigh-muscle	MAAAAVLKQSETQKQLLNQP
PV386791	*E. coli*	*bla*CTX-M-14	Jan-2025	Faium Egypt	Thigh-muscle	MAAAAVLKQSETQKQLLNQP
PV386792	*E. coli*	*bla*CTX-M-15	Jan-2025	Faium Egypt	Swab from-poultry environment	MAVAAVLKKSESEPNLLNQR
PV386793	*E. coli*	*bla*CTX-M-15	Jan-2025	Faium Egypt	Chest-muscle	MAAAAVLKKSESEPNLLNQR
PV386794	*Staphylococcus aureus*	*mec*A	Jan-2025	Faium Egypt	Poultry lung	VEMTERPIKIYNSLGVKDINQ

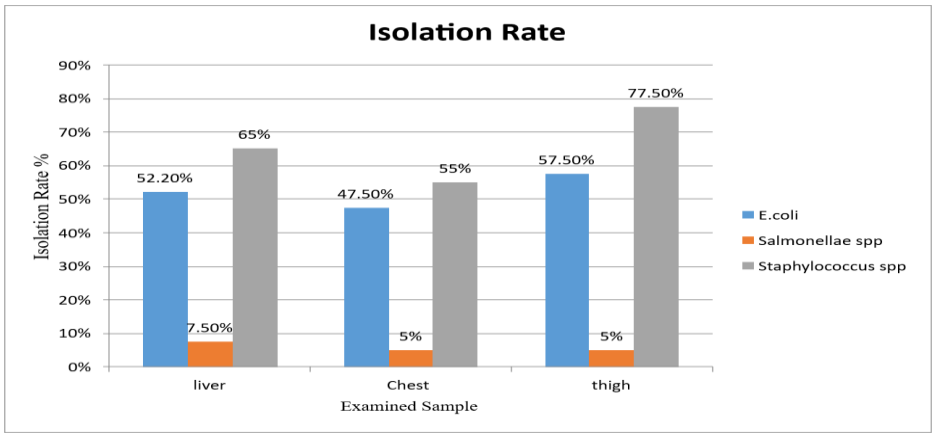

Fig. 1. Isolation Rates of Bacterial Pathogens from Chicken Meat Samples: the isolation rates of three bacterial pathogens—*E. coli*, *Salmonella spp*., and *Staphylococcus spp*.—from different chicken samples (liver, chest, and thigh). The thigh samples showed a significant prevalence of *Staphylococcus spp*. at 77.5%. *E. coli* exhibited the highest isolation rate in liver samples at 52.2%, while *Salmonella spp*. showed lower rates in all sample types.

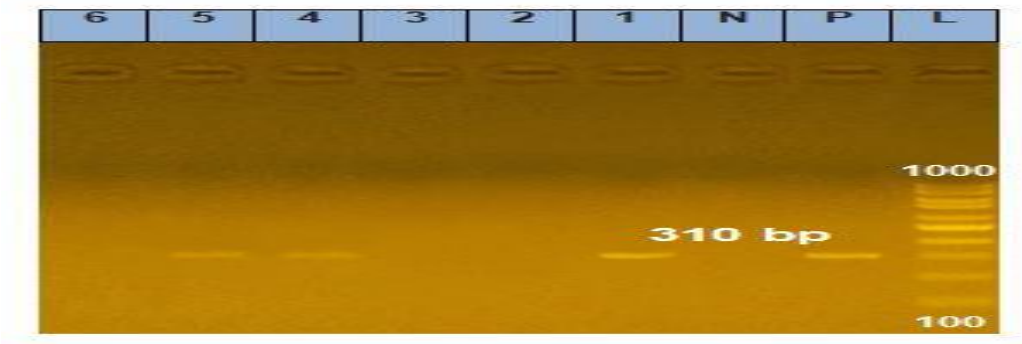

Fig. 2. Agarose gel showing PCR product of *bla*CTX M gene, *E. coli* isolates Lanes 1-6, and *Salmonella* isolates Lanes 7-9 with positive band at 593 bp. L = 100 bp DNA ladder. P = positive control and N = negative control)

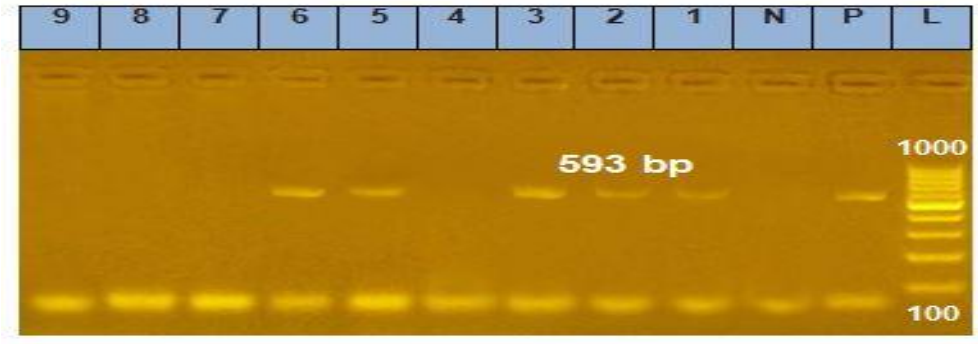

Fig. 3. Agarose gel showing PCR product of *mec*A gene in *S. aureus* isolates Lanes 1-6 with positive band at 310 bp. L = 100 bp DNA ladder. P = positive control and N = negative control.

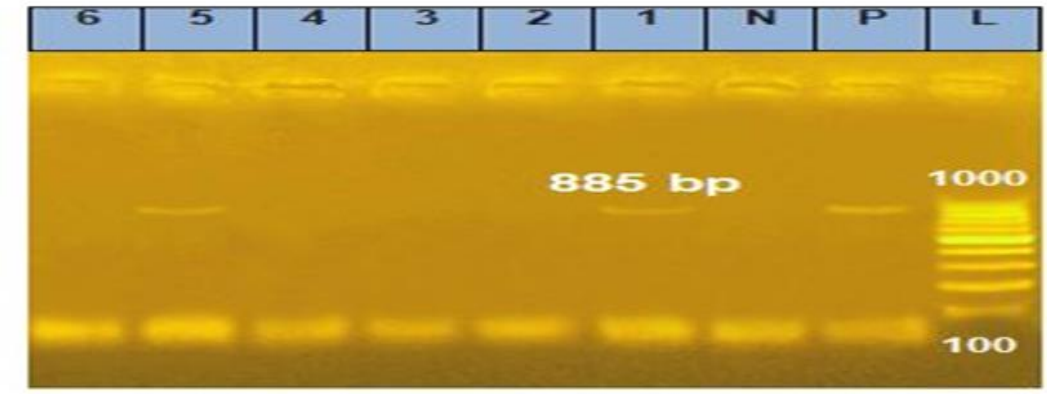

Fig. 4. Agarose gel showing PCR product of *vanA* gene in *S. aureus* isolates Lanes 1-6 with positive band at 885 bp. L = 100 bp DNA ladder. P = positive control and N = negative control.

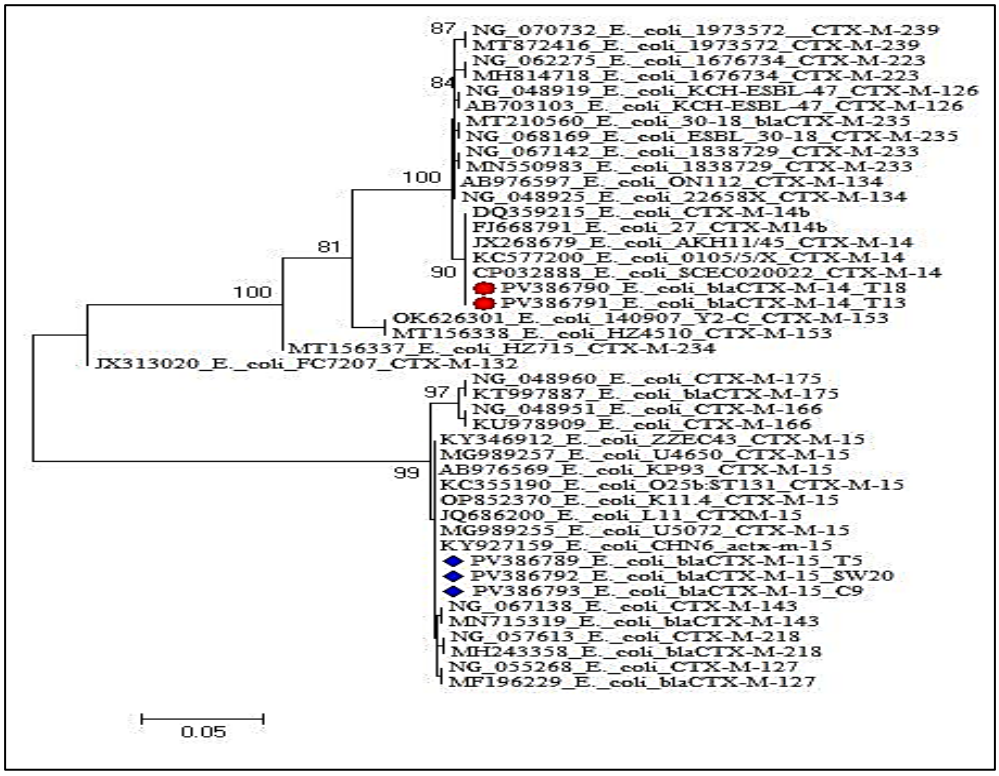

Fig. 5. Phylogenetic tree of various *E. coli* strains and associated gene encoding extended-spectrum beta-lactamase (ESβL) enzymes (*bla*CTX-M) genes; the red dots and blue rhombus indicate strains under study. The partial nucleotide sequences from different strains of *E. coli* were obtained by an Applied Biosystems 3130 genetic analyzer (HITACHI, Japan), and a BLAST® analysis was initially performed to establish sequence identity to GenBank accessions. The phylogenetic tree was created by the Meg Align module of Laser gene DNA Star version 12.1, and phylogenetic analyses were done using maximum likelihood, neighbor joining, and maximum parsimony in MEGA7.

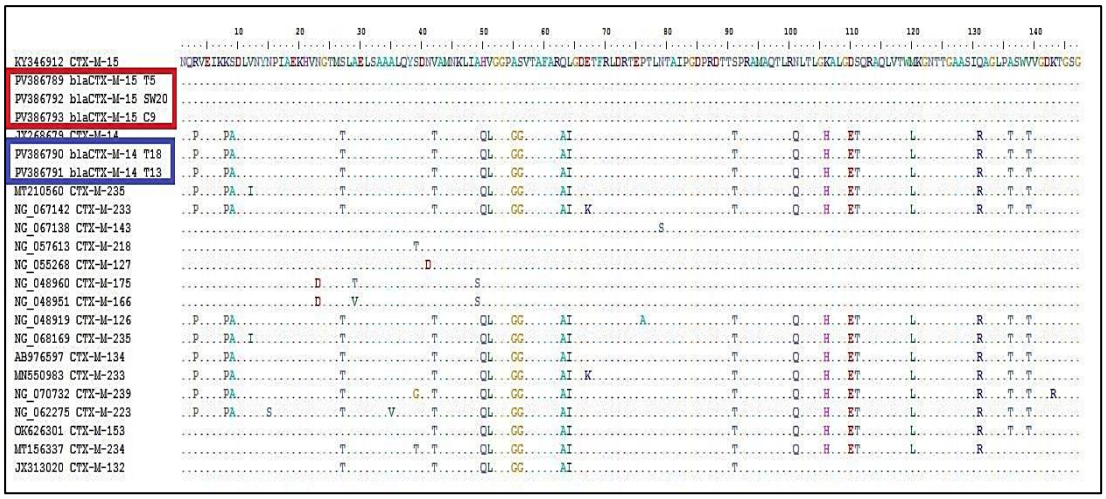

Fig. 6. Shows the percentage of identicality and divergence between various *E. coli* strains according to *bla*CTX-M sequences. The high percentage of identicality (e.g., 100.0%) indicates identical or nearly identical sequences, while lower percentages suggest greater divergence. The highlighted rows and columns (red and blue boxes) likely indicate sequences of interest for comparison. The red box highlights sequences related to *bla*CTX-M-15 and *bla*CTX-M-15 T5, SW20 and C9. The blue box highlights *bla*CTX-M-14 related sequences, including specific variants like *bla*CTX-M-14 T18 and T13.field, and strains available on GenBank.

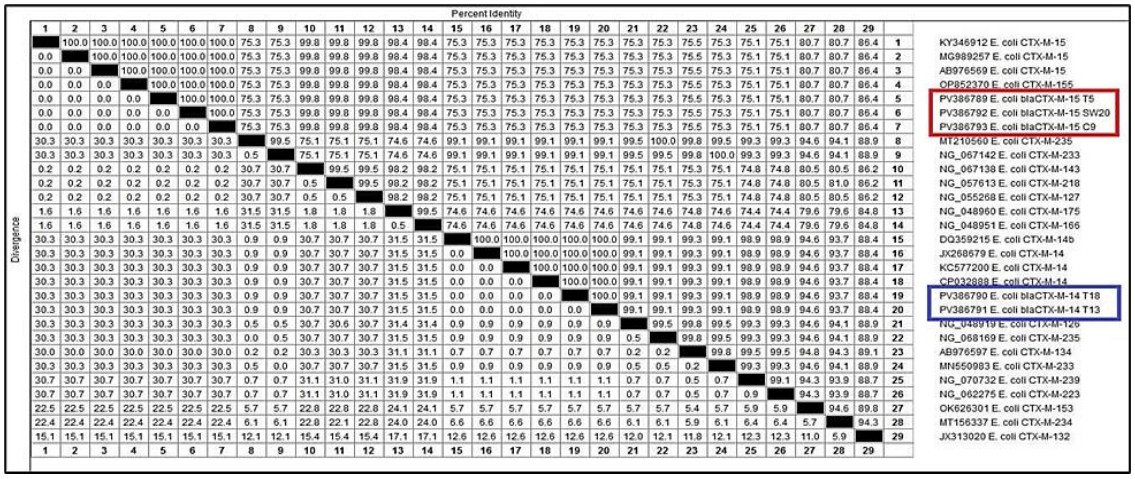

Fig. 7. Multiple sequence alignment of different *CTX*-M beta-lactamase variants is shown, emphasizing amino acid variations at particular locations. The reference sequence, represented by the first sequence (KY346912 *CTX*-M-15), displays the complete amino acid sequence. In the alignment, letters indicate amino acid substitutions at those positions, and dots indicate amino acids that are identical to those in the reference sequence. The strains under this study represented by *CTX*-M-15 variants (PV386789, PV386792, and PV386793) that exhibit a high degree of similarity to the reference are highlighted in the red box. The blue box highlights the *CTX*-M-14 variants (PV386790, PV386791) to illustrate their differences from the *CTX*-M-15 group.

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انتشار ومقاومة مضادات الميكروبات للبكتيريا المنقولة بالغذاء المعزولة من لحوم الدجاج في الأسواق المحلية

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الملخص

تُمثل مسببات الأمراض المنقولة بالغذاء في منتجات الدواجن مصدر قلق كبير على الصحة العامة، لا سيما مع تزايد انتشار مقاومة مضادات الميكروبات. هدفت هذه الدراسة إلى تقييم حدوث وأنماط مقاومة مضادات الميكروبات وجينات المقاومة لبكتيريا الإشريشيا كولاي (*Escherichia coli*) والسالمونيلا (*Salmonella spp*) والمكورات العنقودية *(Staphylococcus aureus)* المعزولة من لحوم الدجاج والبيئه من الاسواق المحليه في محافظة الفيوم، مصر. جُمعت 120 عينة وفُحصت بكتيريًا، تلاها اختبار حساسية مضادات الميكروبات والكشف عن جينات المقاومة المختارة بواسطة تفاعل البلمرة المتسلسل .(PCR) كشفت النتائج عن معدلات انتشار بلغت 57.3% لبكتيريا الإشريشيا كولاي، و5.8% لبكتيريا السالمونيلا، و29.2% لبكتيريا المكورات العنقودية. أظهرت جميع العزلات مقاومة متعددة للأدوية، حيث أظهرت الإشريشيا كولاي مقاومة عالية للأمينوغليكوزيدات، وبيتا لاكتامز، والتتراسيكلين، والفلوروكينولونات. كما أظهرت سلالتا السالمونيلا والمكورات العنقودية الذهبية مقاومة لمعظم المضادات الحيوية المختبرة. أظهر تحليل (PCR) جينات*blaCTX-M* في 83.3% من معزولات الإشريشيا كولاي ، و*mecA* في 50%، و*vanA* في 33.3% من المكورات العنقودية ، مما يؤكد انتشار الإشريكية القولونية المنتجة لـESβL ، والمكورات العنقودية المقاومة للميثيسيلين (MRSA)، والمقاومة للفانكومايسين.(VRSA)

أكدت نتائج التسلسل الجيني (Sequencing) وجود خمس سلالات من الإشريشيا كولاي تحمل الجينين-M CTX- *bla*14و15-M-CTX-*bla*، بالإضافة إلى سلالة واحدة من المكورات العنقودية تحمل جين .*mec*A

كشف phylogenetic tree analysis لسلالات الإشريشيا كولاي المعزولة عن وجود صلة جينية عالية بين المعزولات.

تؤكد نتائج هذا العمل على الحاجة الملحة لتشديد اللوائح المتعلقة باستخدام مضادات الميكروبات في إنتاج الدواجن، وتحسين الممارسات الصحية في الأسواق المحلية، والمراقبة المستمرة للحد من انتشار مسببات الأمراض المقاومة وحماية صحة المستهلك.

الكلمات الدالة: البكتيريا المنقولة بالغذاء، مقاومة الميكروبات، جينات المقاومة، الأمن الغذائي، تسلسل الجينات.