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# Molecular characterization of antibiotic resistance patterns among gram-negative bacteria isolated from red meat

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**Abstract**
The primary objective of this study was to extract *E. coli* samples from red meat samples and analyze their sensitivity to antibiotics. Additionally, the study aimed to investigate the impact of various concentrations of plant extract, as well as the effects of heat and UV radiation on *E. coli* samples. To detect enterotoxins and antimicrobial genes, a PCR assay was employed.
A total of 162 samples of red meat were analyzed using biochemical reactions (microbat kits), resulting in the identification of 63 samples containing *E. coli*. (38.8%).
Cefoperazone exhibits a notable sensitivity rate of 94.1% and a minimal resistance rate of 5.9%, suggesting its potential efficacy against the examined dietary samples. Ampicillin/Sulbactam and Meropenem, two antibiotics, have relatively low sensitivity percentages (41.1% for Ampicillin/Sulbactam and 41.1% for Meropenem) compared to other antibiotics. In addition, Ampicillin/Sulbactam and Meropenem exhibit higher resistance rates, with both showing a proportion of 58.9%. This suggests possible constraints in the effectiveness of these antibiotics against the examined dietary samples, with a notable portion showing resistance.
The plant extract has an effect on the growth of *E. coli*, but it does not have an effect on the enterotoxin genes (*fliC, stx1, stx2, eae*, and *rfbE* genes). However, it does operate as a natural antimicrobial agent, inhibiting the growth of *E. coli*.
The study demonstrates that heat and UV radiation have distinct effects on the detection of virulence genes, leading to alterations in gene expression and DNA integrity. Specific genes, such as *eae* and *rfbE*, exhibit tolerance to certain stresses, whilst others, like *stx-1* and *fliC*, are more sensitive to being affected.

**Keywords**: *Escherichia coli* infections, Virulence genes, Heat and UV radiation effects, Gene expression, Plant extracts, Shiga toxin.

## 1. Introduction

Red meat infections are a substantial public health risk due to their potential to cause foodborne diseases and outbreaks. Red meat, such as beef, lamb, and pork, can become contaminated with different types of harmful microorganisms at different phases of the production and storage process. Escherichia coli (*E. coli*) is particularly notable among these pathogens since it is widespread and has the ability to cause severe infections. Red meat can be contaminated by various bacteria, viruses, and parasites (Shah et al., 2024). The most common bacterial pathogens are the Shiga toxin-producing strains (STEC) such as *E. coli* O157, which can result in severe diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome. *Salmonella* is another pathogen that causes salmonellosis, leading to symptoms like diarrhea, fever, and abdominal cramps. *Listeria monocytogenes* causes listeriosis, which can be particularly severe in pregnant women, newborns, elderly individuals, and those with weakened immune systems. *Campylobacter* is responsible for campylobacteriosis, which is characterized by diarrhea, cramping, and fever (Bodie et al., 2023). Consuming infected red meat can have a range of health effects, from moderate gastrointestinal discomfort to severe, life-threatening illnesses such as *E. coli* infections. Common symptoms encompass intense abdominal pains, diarrhea (often accompanied by blood), and vomiting. In more extreme instances, it might result in hemolytic uremic syndrome (HUS), a condition that can lead to renal failure (Shah et al., 2024). Salmonella infections Common symptoms encompass diarrhea, fever, and abdominal cramps. In severe instances, it has the potential to result in invasive infections, including bloodstream infections (Ali & Alsayeqh, 2022). The symptoms of listeriosis encompass fever, myalgia, and gastrointestinal problems. It has the potential to result in serious consequences, such as septicemia and meningitis. Campylobacteriosis presents with symptoms such as diarrhea, cramps, and fever. Severe instances can result in Guillain-Barré syndrome, an uncommon condition characterized by the immune system's assault on the nerves (Schlech, 2019).

*Escherichia coli* is a prevalent and typically benign bacterium found in the human intestinal flora. Five types of *E. coli* causing diarrhea in humans and other warm-blooded animals have been identified (Harakeh et al., 2005). The types of *E. coli* that are included are enterotoxigenic *E. coli (ETEC), enteropathogenic E. coli (EPEC), enteroaggregative E. coli (EAEC)*, enter invasive *E. coli (EIEC),* and enterohaemorrhagic *E. coli (EHEC)* (Ramos et al., 2020). The latter refers to Shiga Toxin *(Stx)*-Producing *E coli (STX-EC)*. Shiga toxin *(Stx)*-producing *E. coli* (STX-EC), also referred to as Vero toxin-producing *E. coli* is linked to newborn diarrhea, hemorrhagic colitis, thrombotic thrombocytopenic purpura, and hemolytic uremic syndrome in adults (Joseph et al., 2020). The occurrence of *E. coli* infection in people is known as *E. coli* syndrome. *E. coli O157:H7*, a member of the STX-EC group, is the predominant serotype seen in persons with hemorrhagic colitis. Instances of infection caused by those bacteria have arisen as a result of consuming animal-derived food products that have been contaminated (Phanindranath Reddy Fifth Year BVSc et al., 2021). Studies indicate that *E. coli O157:17* infections are commonly seen in cattle, goats, sheep, and other livestock. In the study conducted by (Harakeh et al., 2006), it was found that cattle are the main carriers of this virus, accounting for 34 animals in total. Nevertheless, researchers have found five distinct types of *E. coli* that are responsible for causing diarrhea in both humans and other warm-blooded animals (Nataro & Kaper, 1998). The types of *E. coli* bacteria that are included are enterotoxigenic *E. coli (ETEC),* enteropathogenic *E. coli (EPEC),* enteroaggregative *E. coli (EAEC)*, enter invasive *E. coli (EIEC),* and enterohaemorrhagic *E. coli (EHEC)* (Jafari et al., 2012). The latter refers to Shiga Toxin (*Stx*)-Producing *E. coli (STX-EC)*. Shiga toxin (*Stx*)-producing *E. coli (STX-EC)*, often referred to as Vero toxin-producing *E. coli*, is linked to several health conditions in humans including newborn diarrhea, hemorrhagic colitis, thrombiotic thrombocytic purpura, and hemolytic uremic syndrome (Paton & Paton, 1998). *E. coli O157:H7*, a member of the STX-EC group, is the predominant serotype seen in persons with hemorrhagic colitis *(E. coli O157:H7)*. Instances of infection caused by these bacteria have arisen as a result of consuming animal-derived food products that have been contaminated (Gambushe et al., 2022). Studies indicate that *E. coli O157:17* infections are commonly seen in cattle, goats, sheep, and other livestock.

Antibiotics are a category of antimicrobial substances that have significantly transformed the management of bacterial illnesses and have been important in saving numerous lives since their initial identification. These potent chemicals can specifically target and restrict the growth or eliminate bacterial infections while causing minimal damage to human cells (Chen et al., 2023). *E. coli* can develop antibiotic resistance either through spontaneous mutations or by acquiring resistance genes by horizontal gene transfer processes, such as conjugation, transformation, or transduction. Resistance mechanisms may exist on plasmids or be integrated into the bacterial chromosome, enabling resistance to several drugs and resulting in the development of multidrug-resistant (MDR) strains (Liu et al., 2024).

The plant extracts of clove, cinnamon, zaatar, artemisia, and rosemary exhibit antimicrobial properties against *E. coli*, particularly against resistant strains. These extracts exert their effect on *E. coli* by disrupting the bacterial cell membrane and inhibiting the activity of bacterial enzymes involved in essential metabolic pathways, such as the respiratory chain (Chassagne et al., 2021). Moreover, these plant extracts are effective natural preservatives due to their antimicrobial and antioxidant activities. This delays the shelf life of food products and preserves their quality. *Escherichia coli* exhibits the heat shock response, which leads to an upregulation in the synthesis of heat shock proteins that function as molecular chaperones (Singh et al., 2024).

The membrane undergoes increased fluidity and permeability, allowing heat to induce cell death by protein denaturation and membrane damage (Li & Gänzle, 2016). UV exposure can cause fatal DNA damage in *E. coli* by forming pyrimidine dimers and strand breaks. This hinders the process of replicating and utilizing. UV exposure induces the production of reactive oxygen species, which harm proteins. The combination of DNA damage and cellular oxidation is lethal to bacteria (Kciuk et al., 2020). After subjecting the sample to heat and UV light, we performed the PCR assay to detect enterotoxins.

Our observations indicate the absence of the genes *Stx 1* and *FliC*. The *stx 1* gene is an essential factor in the pathogenesis and virulence of bacterial pathogens, specifically Shiga toxins, which are classified into two primary classes. The main role of *Stx1* is to limit protein synthesis in eukaryotic cells,

resulting in cellular death (M. S. Lee et al., 2016).

The *FliC* gene is responsible for producing the flagellin protein, which serves as the main building block of the bacterial flagellum. The main task of the *FliC* gene is to serve as the structural element for the flagellar filament, allowing for movement. However, its significance goes beyond this function, as it also plays a part in other areas of bacterial physiology, pathogenicity, and interactions with the environment and host species (Nedeljković et al., 2021).

## 2. Material and Methods

### 2.1. Sample collection

In this study, a total of 162 retail samples including (27 minced meat), (27 koftas), (27 burgers), (27 sausages), (27 sauces) and (27 Lanchow) were collected randomly from various shops and butchers in Tanta Gharbia. All the samples represented by cubed raw meat were non-frozen and kept at refrigeration condition (+ 4 c) (Richardson, 2022). The samples were directly transported to a laboratory without opening the package and all collected samples were placed in portable insulated cold boxes.

### 2.2. Isolation and identification of *E. coli* from samples

The raw red meat samples were tested for isolating *E. coli* typically involves culturing samples on selective media such as MacConkey agar media which inhibits the growth of other bacteria and allows *E. coli* to grow and identify colonies by using biochemical tests and microbact strip assay for identification of E. coli isolates (Lupindu & Lupindu, 2017).

By adding 10 grams of samples in a sterile mortar then grind with 90 ml of buffered peptone water for 5 minutes. after homogenization, we used a sterile swape to spread the samples across the surface of the MacConkey agar plate. Incubate the plates in the incubator at 37 c for 24 hours then after incubation observe the plates' lactose fermenting bacteria will produce pink colonies. Also, the colonies isolated from positive cultures were investigated to determine the biochemical characteristics of *E. coli.* This included examining characteristics such as gram staining, pigment production, colony morphology, oxidase production capability, and Microbact biochemical kites (Oxoid, n.d.) then transferring the colonies to nutrient agar media and incubating to make antibiotic test.

### 2.3. Antibiotic susceptibility test (disk diffusion method)

All verified *E. coli* strains performed antibiotic susceptibility testing utilizing the disc diffusion method on Muller agar. The agar used is Hinton agar, which is manufactured by Merck in Germany.

A study was done to assess the sensitivity of 60 strains of *E. coli* to 10 different types of antimicrobials from several classes of antibiotic discs. Gentamycin (Cn), nitrofurantoin (F), cefoperazone/sulbactam (Ces), ciprofloxacin (Cip), piperacillin/tazobactam (Tzp), ampicillin/sulbactam (Sam), meropenem (Mem), cefoperazone (Cep), norfloxacin (Nor), and amoxicillin clavulanic acid (Amc). Place the antibiotic discs, which contain a known concentration of antibiotics, on the surface of the plates that have been inoculated with *E. coli*. Incubate the plates at a temperature of 37°C for 24 hours (Jan Hudzicki, 2009). Following the incubation period, the sizes of the zones of inhibition on the plates were determined by employing a clear ruler and documenting the results.

### 2.4. Effect of plant extract on *E coli*

Extraction of plant extract by Taking (cinnamon, clove, zaatar, artemisia, and rosemary) plants were washed using distilled water and then dried in an oven at 50 C and ground well to a fine powder using a blender, then prepare an extraction using zaatar, cinnamon, clove, artemisia, and rosemary. Combine 50 grams of plant powder with 200 ml of methanol solvent in a sterile flask. Transfer the flask containing the powder into a shaker and allow it to dissolve in the solvent for 24 hours. Subsequently, filter the solution using filter paper, followed by centrifugation at a speed of 9000 rpm for 10 minutes. Finally, pass the solution through a Whatman filter paper to get a purified filtrate. The solvent was eliminated through evaporation.

The extract obtained was subsequently dried, weighed, and stored at a temperature of 4°C until it was ready for use.

A biological assay is a scientific method used to measure and evaluate the biological activity or potency of a substance.

Mix 100 grams of plant extract with 1 ml of DMSO (dimethyl sulfoxide) and dissolve them together. Also, mix 50 grams of plant extract with 1 ml of DMSO and dissolve them together.

To begin, dissolve 25 grams of plant extract in 1 ml of DMSO. Next, prepare nutrient media and inoculate it with E. coli samples. Then, create 6 wells on plates and add 100 microns of plant extract (zaatar, cinnamon, clove, artemisia, and rosemary) to each well. Use DMSO as a control in one of the wells. Repeat

this process for three different concentrations of plant extract. Incubate the plates in a 37°C incubator for 24 hours. Finally, observe the results by measuring the diameter of the inhibition zone using a clear ruler and record the findings (In et al., 2006).

**2.5. DNA extraction and PCR analysis**

After cultivation on McConkey agar, the isolated *E. coli* colonies were transferred to 5 mL of Luria-Bertani media and cultured at 37°C overnight. The virulence gene of E. coli was successfully identified using a PCR experiment that utilized the primer pair consisting of *Sxt1, sxt2, eae, filC*, and *rfb* genes. The primer sequences used for genetic profiling of *E. coli* exposed to a plant extract, as well as the identification of five virulence genes, are provided in Table 1. Following amplification, the resulting products are subjected to electrophoresis on a 1% agarose gel that has been dyed with a safe dye. A defined band detected after DNA amplification suggested a positive outcome.

**Data analysis:**

A gel documentation system (Geldoc-it, UVP, England), was applied for data analysis using Totallab analysis software, ww.totallab.com, (Ver.1.0.1).

**Table(1)** Specific primer sequence under study.

| Genes | Sequences | Amplicon size (bp) | References | Gene function |
|---|---|---|---|---|
| ***fliC*** | AGC TGC AAC GGT AAG TGA TTT GGC AGC AAG CGG GTT GGT C | 949 | (Wang et al., 2000) | Synthesis of flagella allowing *E. coli* to move |
| ***stx1*** | TGT CGC ATA GTG GAA CCT CA TGC GCA CTG AGA AGA AGA GA | 655 | (Bai et al., 2010) | Inhibit protein synthesis in host cell leading to cell death |
| ***stx2*** | CCA TGA CAA CGG ACA GCA GTT TGT CGC CAG TTA TCT GAC ATT C | 477 | (Fagan et al., 1999) | Inhibit protein synthesis like *stx 1* |
| ***eae*** | CAT TAT GGA ACG GCA GAG GT ACG GAT ATC GAA GCC ATT TG | 375 | (Bai et al., 2010) | Formation of attaching and effacing lesions during infection |
| ***rfbE*** | CAG GTG AAG GTG GAA TGG TTGTC TTA GAA TTG AGA CCA TCC AATAAG | 296 | (Bertrand & Roig, 2007) | Biosynthesis of o-antigen component of bacterial lipopolysaccharide molecule of the outer membrane |

**2.6. Heat Experiments**

Inoculate a colony of *E. coli* onto a sterile glass plate containing MacConkey media. Incubate the glass plate in an incubator at a temperature of 37°C for a duration of 24 hours. Following incubation, subject the glass plate to a temperature of 70°C in an oven for a period of 2 hours. Finally, observe the colonies of E. coli that remain viable through DNA purification and PCR (Arsène et al., 2000).

**2.7. Ultraviolet Irradiation Experiments**

Inoculate a colony of *E. coli* onto sterile plates containing MacConkey media. Incubate the plates in an incubator at a temperature of 37°C for a duration of 24 hours. After incubation, subject the plates to a low-pressure collimated beam apparatus to conduct UV disinfection experiments. Expose the plates to light emitted by a 15-watt mercury vapor lamp with a wavelength of 254 nm positioned above the cells in the plate for a period of 2 hours.

Utilize a radiometer with a 254 nm detector to accurately gauge the intensity of UV light and verify appropriate amounts of irradiation. After exposing the plates to UV radiation, examine them to evaluate whether *E. coli* colonies remain alive or whether there is a decrease in colony count, suggesting the effectiveness of UV disinfection. Perform PCR amplification and detection of virulence genes to assess the effect of UV irradiation on the pathogenicity of *E. coli* and the expression of virulence genes (Varna Kodoth, 2015).

## 3. RESULT

### 3.1. prevalence of *E coli* in red meat

The total samples of red meat are 162 collected from various markets and butchers in Tanta-Gharbia. Samples cultured on selective media (MacConkey media) that produced 63 samples of E coli (Figure 1) identified by microbat kits and biochemical tests are shown in Table (2)

| Samples (162) | Samples no. (162) | E. coli no. (63) | Percentage % |
|---|---|---|---|
| **Minced meat** | **27** | **12** | **44.4%** |
| **kofta** | **27** | **11** | **40.7%** |
| **burger** | **27** | **9** | **33.3%** |
| **sausage** | **27** | **9** | **33.3%** |
| **sauces** | **27** | **10** | **37%** |
| **Lanchow** | **27** | **12** | **44.4%** |

**Table (2)** In this study, the total samples are (162) and produced (63) samples of *E coli* so (38.8) of the samples are E coli.

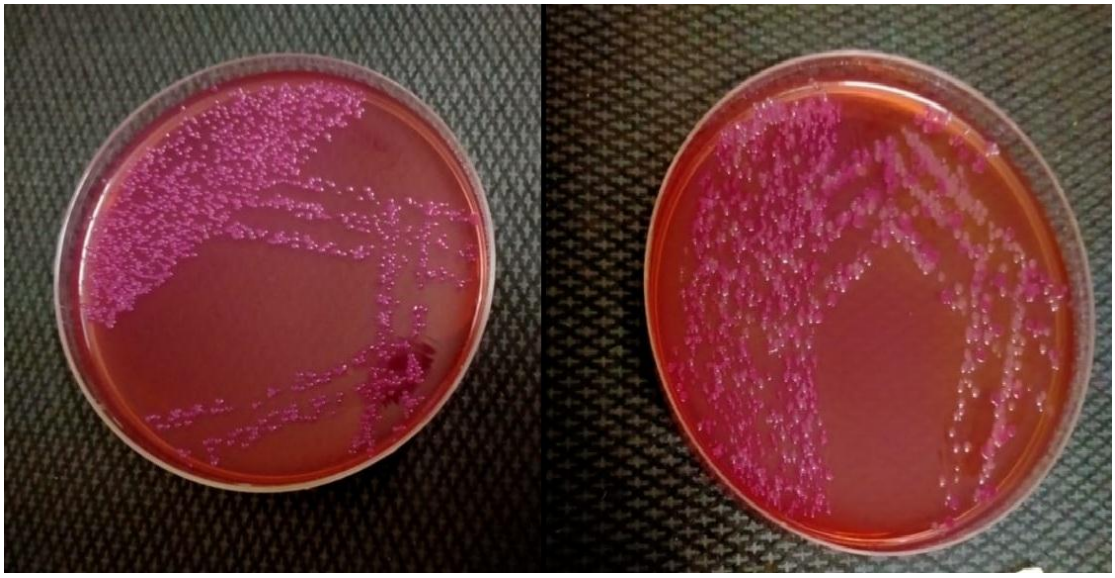


**Fig. (1)** Show culturing of *E. coli* on MacConkey media

### 3.2. Biochemical Tests and Identification of *E. coli*

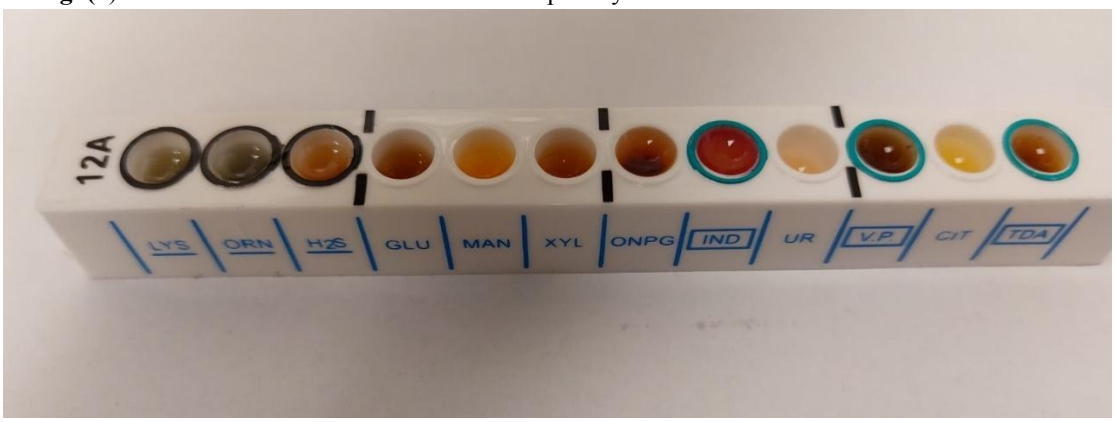
The biochemical tests and identification of *E. coli* by Microbact kits (Oxoid, n.d.) are shown in **Table 3** *Escherichia coli (E. coli)* is Gram-negative and rod-shaped, it can ferment sugars, produces catalase, and exhibits motility via flagella. The Microbact (Gram-negative kits) is to be used for the identification of E. *coli* (**Figure 2**).

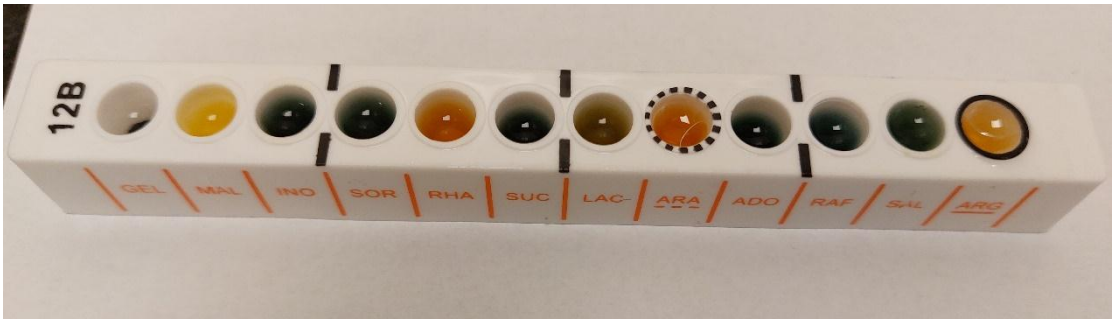
**Table (3)** The basic properties and identification of *Escherichia coli* samples in the study.

| Basic Characteristics | Properties (*Escherichia coli*) | Negative | Positive |
|---|---|---|---|
| **Gram staining** | Gram negative bacilli | | |
| **Shape** | Rods | | |
| **Motility** | Motile | | |
| **Spore** | Non-Sporing | | |
| **Flagella** | Flagellated | | |
| **Catalase** | Positive | No bubbles produced | bubbles produced |

| | | | |
|---|---|---|---|
| **Oxidase** | Negative | Colourless | Blue |
| **Lysine** | Positive | Yellow | Blue-green |
| **Ornithine** | Positive | Yellow-green | Blue |
| **H2S** | Negative | Straw colour | Black |
| **Glucose** | Positive | Blue-green | Yellow |
| **Mannitol** | Positive | Blue-green | Yellow |
| **Xylose** | Positive | Blue-green | Yellow |
| **ONPG** | Positive | Colourless | Yellow |
| **Nitrate** | Positive | Yellow colour | Red colour |
| **Indole** | Positive | Colourless | Pink-red |
| **Urease** | Negative | Straw colour | Pink-red |
| **VP** | Negative | Straw colour | Pink-red |
| **Citrate** | Negative | Green | Blue |
| **TDA** | Negative | Straw colour | Cherry red |
| **Gelatin** | Negative | Colourless | Black |
| **Malonate** | Negative | Green | Blue |
| **Inositol** | Negative | Blue-green | Yellow |
| **Sorbitol** | Positive | Blue-green | Yellow |
| **Rhamnose** | Positive | Blue-green | Yellow |
| **Sucrose** | Negative | Blue-green | Yellow |
| **Lactose** | Positive | Blue-green | Yellow |
| **Arabinose** | Positive | Blue-green | Yellow |
| **Adonitol** | Negative | Blue-green | Yellow |
| **Raffinose** | Negative | Blue-green | Yellow |
| **Salicin** | Negative | Blue-green | Yellow |

**Fig. (2)** Results of simulated MICROBACT strip assay for identification ***Escherichia coli*** isolate.






**3.3. Antibiotic sensitivity test with *E coli***

The results of antibiotic susceptibility testing conducted on *E coli* samples (63) including minced meat (12), kofta (11), burger (9), sausage (9), sauces (10), and Lanchow (12) we used (10) antibiotic discs with known concentration by the disc diffusion method (Figure 4). In addition, the percentage of isolates resistant (% total Resistant/total) and sensitive (% total Sensitive/total) to each antibiotic tested is provided for each sample type and this is shown in **Table 4**.

The sensitivity percentage of cefoperazone is 94.1%, while its resistance percentage is 5.9%. This suggests that cefoperazone has the potential to be successful against the examined food samples. The antibiotics Gentamycin, Nitrofurantoin, Ciprofloxacin, Piperacillin/Tazobactam, and Cefoperazone/Sulbactam show different levels of effectiveness in the food samples. Gentamycin, Nitrofurantoin, and Piperacillin/Tazobactam have higher sensitivity percentages, ranging from 52.9% to 76.4%. Similarly, Ciprofloxacin and Cefoperazone/Sulbactam also demonstrate higher sensitivity, ranging from 58.8% to 64.7%. These findings indicate that these antibiotics have the potential to be successful treatments for a significant proportion of the food samples that were analyzed.

Norfloxacin and Amoxicillin/Clavulanic Acid, two types of antibiotics, have shown moderate sensitivity rates (58.8% for Norfloxacin and 64.7% for Amoxicillin/Clavulanic Acid), but also very significant resistance rates (41.2% for Norfloxacin and 35.3% for Amoxicillin/Clavulanic Acid). This indicates that although these antibiotics may be successful in treating certain strains, a significant fraction of the food samples that were examined show resistance.

Ampicillin/Sulbactam and Meropenem, both antibiotics demonstrate relatively reduced sensitivity rates (41.1% for Ampicillin/Sulbactam and 41.1% for Meropenem) compared to other antibiotics. Furthermore, Ampicillin/Sulbactam and Meropenem exhibit a higher percentage of resistance, with both drugs showing a resistance rate of 58.9%. This suggests that these antibiotics may not be very effective against the food samples that were analyzed, as a considerable fraction of them showed resistance.

In general, the data demonstrate diverse antibiotic susceptibility patterns among the food samples that were analyzed (**Figure 3**). While many drugs exhibit encouraging rates of sensitivity, others display notable resistance, underscoring the intricate nature of antibiotic resistance in foodborne bacteria. These findings emphasize the significance of cautious antibiotic usage in food production and the necessity for ongoing monitoring of antibiotic resistance to inform effective treatment approaches and reduce the transmission of resistant microorganisms in the food chain.

| Antibiotic disc | Minced meat | Kofta | Borger | Sausage | Sauces | Lanchow | Total. Sensitive/ Total samples | Total. Sensitive/ Total samples % | Total. resistant/Total samples | Total. resistant/Total samples % |
|---|---|---|---|---|---|---|---|---|---|---|
| Gentamycin (10mg) | 12/12 | 11/11 | 0/9 | 4/9 | 6/10 | 0/12 | 33/63 | 52.3% | 30/63 | 47.6% |
| Nitrofurantoin (300mg) | 12/12 | 11/11 | 0/9 | 8/9 | 10/10 | 7/12 | 48/63 | 76.1% | 15/63 | 23.8% |
| Cefoperazone/sulbactam (75/30) | 9/12 | 11/11 | 0/9 | 6/9 | 10/10 | 4/12 | 40/63 | 63.4% | 23/63 | 36.5% |
| Ciprofloxacin (5mg/disc) | 12/12 | 11/11 | 0/9 | 4/9 | 10/10 | 0/12 | 37/63 | 58.7% | 26/63 | 41.2% |
| Piperacillin/Tazobactam (30 mg) | 12/12 | 11/11 | 9/9 | 6/9 | 10/10 | 0/12 | 48/63 | 76.1% | 15/63 | 23.8% |
| Ampicillin/Sulbactam (10 mg) | 3/12 | 11/11 | 4/9 | 0/9 | 0/10 | 8/12 | 26/63 | 41.2% | 37/63 | 58.7% |
| Meropenem (10mg) | 12/12 | 11/11 | 0/9 | 0/9 | 10/10 | 3/12 | 26/63 | 41.2% | 37/63 | .58.7% |
| Cefoperazone (75mg) | 12/12 | 11/11 | 9/9 | 9/9 | 10/10 | 11/12 | 54/63 | 85.7% | 9/63 | 14.2% |
| Norfloxacin (10mg) | 0/12 | 11/11 | 9/9 | 8/9 | 10/10 | 0/12 | 37/63 | 58.7% | 26/63 | 41.2% |
| Amoxicillin/Clavulanic Acid (30mg) | 6/12 | 8/11 | 9/9 | 6/9 | 5/10 | 7/12 | 41/63 | 65% | 22/63 | .34.9% |

**Table (4)** Antibiotic sensitivity test with *E coli.*

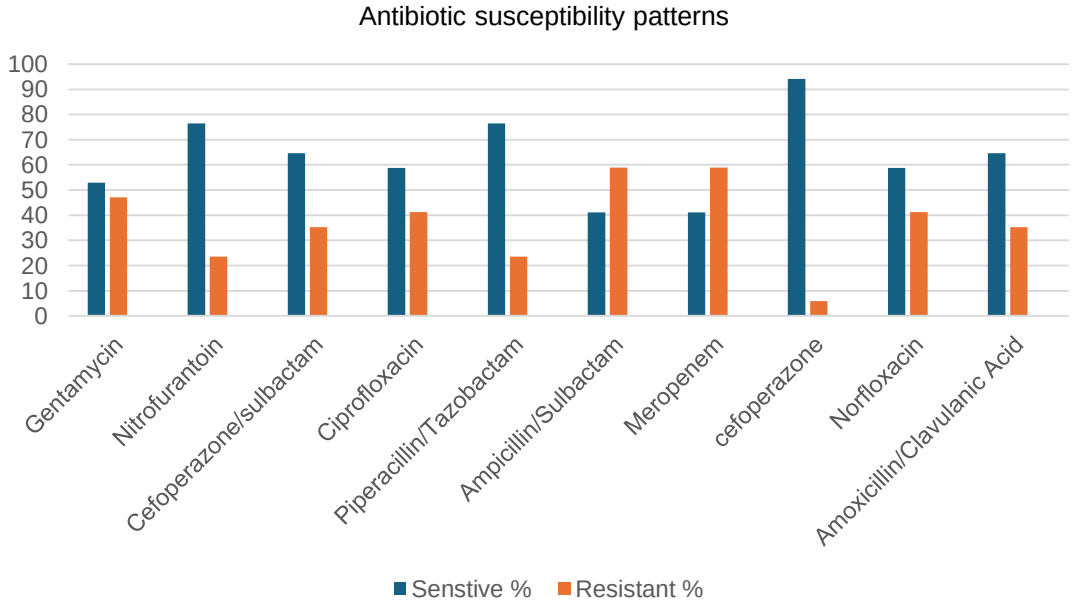



**Fig. (3)**Antibiotic susceptibility patterns

**Fig. (4**) Antibiotic susceptibility by disc diffusion method

### 3.4. Effect of plant extract on *E. coli* samples

In our study, we utilized the most highly resistant samples of *E. coli* for the antibiotic disc (17) samples. We then inoculated these samples onto nutrient agar medium by creating a well on the agar. Next, we added the plant extract to the well and proceeded to incubate the samples. The effect of the plant extract at three different concentrations. An evident concentration-dependent effect is detected in all plant extracts. As the concentration of the substance increases from 25 mg to 100 mg, the minimum inhibitory concentration (MIC) often lowers, suggesting a more potent inhibitory effect. The declining pattern in minimum inhibitory concentration (MIC), as the concentrations decrease, demonstrates a dose-dependent response of the plant extracts against E. coli isolates (**Figure 5**). Every plant extract has different levels of efficacy against *E. coli* isolates. For instance, Rose Mary consistently exhibits the highest Minimum Inhibitory Concentration (MIC) values across all concentrations and types of meat, indicating that it may be significantly more effective compared to the other extracts. Additionally, when Artemisia extract is applied to minced beef, the minimum inhibitory concentration (MIC) reduces from 16.9 at a dose of 100 mg to 8.3 at 50 mg, and further decreases to 3.9 at a concentration of 25 mg. This pattern is similar in other plant extracts as well. The results repeatedly showed that cinnamon had lower minimum inhibitory concentration (MIC) values, indicating its potential as a very effective antibacterial agent. Zaatar exhibited consistent effectiveness across various concentrations, indicating a persistent antibacterial action. These discoveries have practical consequences for ensuring the safety and preservation of food. Plant extracts, especially when used in greater concentrations, have the potential to act as natural antibacterial agents that can effectively prevent the growth of *E. coli* in meat products.

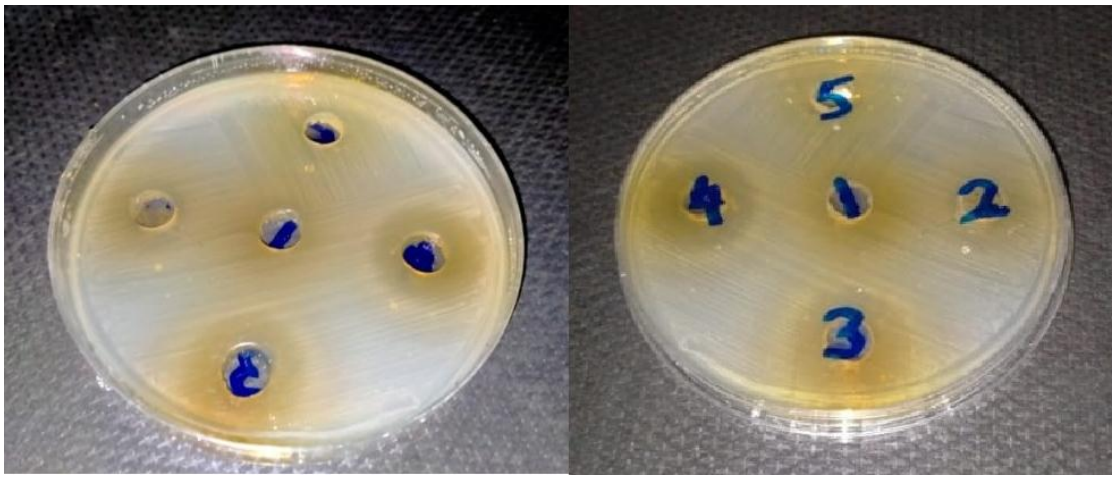



**Fig. (5)** Show the effect of plant extract on *E. coli* samples.

### 3.5. PCR assay for detection of enterotoxins and antimicrobial-resistant genes after exposure of *E. coli* to plant extracts

After screening plant extract on *E coli* samples, we made traditional PCR using five genes *fliC, stx1, stx2*, *eae* and *rfbE* genes were employed as molecular markers to investigate and detect the molecular mechanism of virulence for *E. coli* (**Figure 6**).

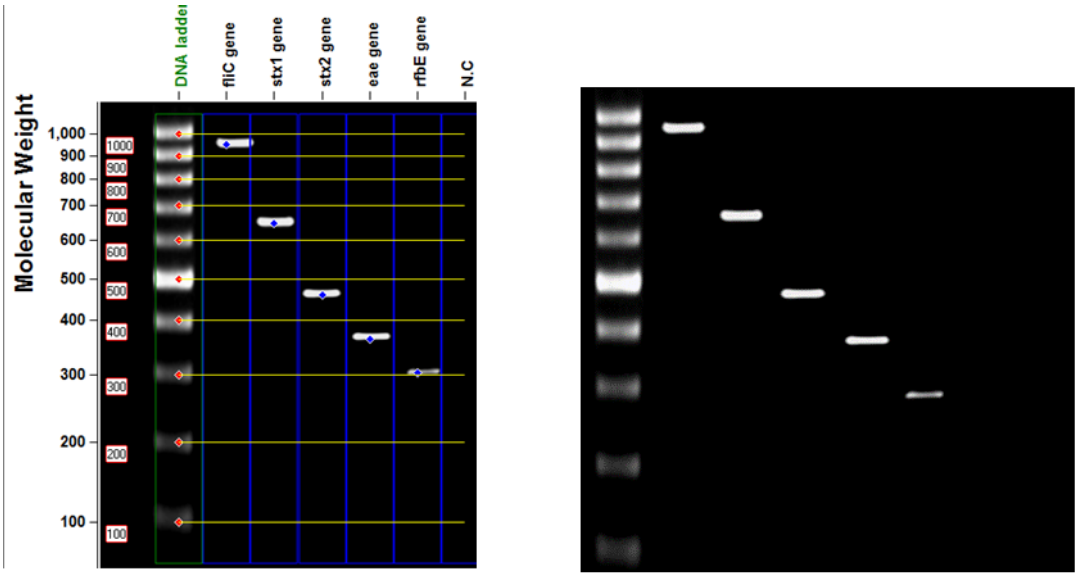



**Fig. (6)** shows Agarose gel electrophoresis of PCR product amplification of specific genomic products and Fragments length calculation for *fliC, stx1, stx2*, *eae* and *rfbE* genes for *E.coli* isolate *fliC* gene in ( M.W: 952 bp ), *sxt1* gene in ( M.W:645 bp), *sxt2* gene in ( M.W:471 bp), *eae* gene in (M.W: 372 bp) and *rfbE* gene in (M.W:296 bp ).

| DNA ladder (1) | | fliC gene (2) | | stx1 gene (3) | | stx2 gene (4) | | eae gene (5) | | rfbE gene (6) | | N.C (7) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lane % | MW | Lane % | MW | Lane % | MW | Lane % | MW | Lane % | MW | Lane % | MW | Lane % | MW |
| 3.39 | 1000.000 | 0.57 | 952.115 | 0.45 | 645.226 | 0.59 | 470.517 | 0.54 | 371.630 | 0.47 | 296.635 | | |
| 0.18 | 900.000 | | | | | | | | | | | | |
| 4.09 | 800.000 | | | | | | | | | | | | |
| 0.42 | 700.000 | | | | | | | | | | | | |
| 14.13 | 600.000 | | | | | | | | | | | | |
| 0.16 | 500.000 | | | | | | | | | | | | |
| 0.31 | 400.000 | | | | | | | | | | | | |
| 12.84 | 300.000 | | | | | | | | | | | | |
| 17.26 | 200.000 | | | | | | | | | | | | |
| 30.70 | 100.000 | | | | | | | | | | | | |

**Table (5)** data parameters for *fliC, stx1, stx2*, *eae* and *rfbE* genes for *E. coli* isolate.

The agarose gel electrophoresis results indicate the presence of several virulence genes in the *E. coli* isolate. Every band observed on the gel corresponds to a distinct DNA fragment, which indicates the presence of a certain gene in the *E. coli* sample. The measured fragment lengths correspond to the expected sizes of the targeted genes: *fliC* (952 bp), *stx1* (645 bp), *stx2* (471 bp), *eae* (372 bp), and *rfbE* (296 bp). The existence of the *fliC* gene suggests the enhanced ability to move and colonize, which could result in greater spreading and pathogenicity. The identification of the stx1 and *stx2* genes is highly significant since they encode significant Shiga toxins, which are responsible for causing serious disorders such as hemorrhagic colitis and HUS. Identifying these genes in strains helps to quickly identify possible public health problems. The *eae* gene is crucial in the development of EPEC and EHEC infections as it facilitates the attachment and strong adherence of *E. coli* to the cells lining the intestines. The *rfbE* gene promotes the diversity of bacterial somatic (O) antigens, which can potentially result in serious gastrointestinal diseases such as hemorrhagic colitis. After applying plant extract to *E. coli*, we detected the presence of five genes, and these genes remained unaffected.

**3.6. PCR assay for detection of enterotoxins and antimicrobial-resistant genes after exposure of *E. coli* isolates to UV and heat.**

The results (Figure 7) show how heat and UV exposure affect the genetic composition of *E. coli*, specifically in the detection of virulence genes. The diagram illustrates the identification of four genes (fliC, *stx-1, eae, and rfbE* ) within the control strain of *E. coli*. Each gene's length is measured in base pairs (bp), with *fliC* being the longest at 949 bp, followed by *stx-1* (655 bp), *eae* (375 bp), and *rfbE* (296 bp).

After subjecting *E. coli* to heat, the presence of the eae gene (375 bp) and the *rfbE* gene (296 bp) is detected in Line 1. Specifically, the absence of the *fliC* and *stx-1* genes in this lineage indicates that heat exposure may influence their expression or detectability. The results from Line 2, in which *E. coli* was subjected to UV radiation, indicated the presence of the *eae* gene (375 bp) and the *rfbE* gene (296 bp), but the *fliC* and *stx-1* genes were not detected. This implies that UV exposure may have affected the expression or detectability of these genes in a manner similar to heat exposure. The study demonstrates the different effects of heat and UV light on the detection of virulence genes, hence affecting gene expression and DNA integrity. Specific genes, such as *eae* and *rfbE*, exhibit resistance to these stresses, while others, like *stx-1* and *fliC*, are more susceptible to their effects. This illustrates the complex correlation between environmental conditions and bacterial gene expression, providing a vital understanding of how *E. coli* adapts and modulates its virulence by its surroundings.

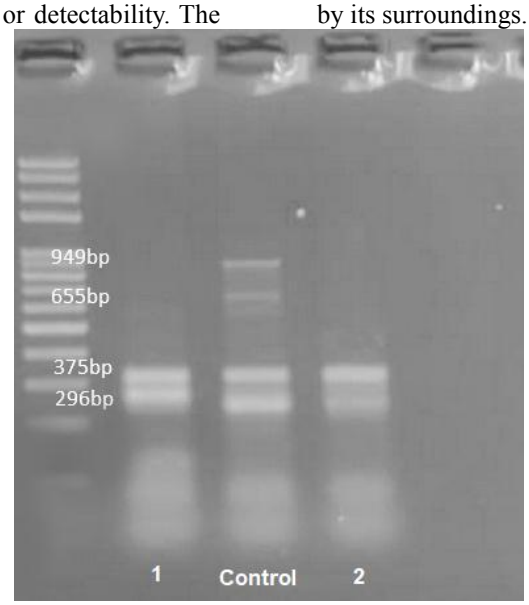



**Fig. (7)** shows the detection of *fliC* gene in (M. W: 949 bp), *sxt-1* gene in (M. W: 655 bp), *eae* gene in (M. W: 375 bp), and *rfbE* gene in (M. W:296 bp) all in *E. coli* control line. The figure also shows the detection of *eae* gene in (M. W:375 bp) and *rfbE* gene in (M. W:296 bp) in line 1 which *E. coli* was

exposed to heat. while detection of *rfbE* gene in (M. W: 296 bp) and *eae* gene in (M. W: 375 bp) all in line 2 which *E. coli* exposed to U.V.

## 3. Discussion

The research examined 162 samples of red meat obtained from several markets and shops in Tanta-Gharbia. The findings showed that *E. coli* was present in 38.8% of the samples. This suggests a notable prevalence of *E. coli* in the nearby meat provision, which is a matter of worry about the safety of food and the well-being of the general population. The differing occurrence rates seen in various meat products, such as minced beef, kofta, burger, sausage, sauces, and Lanchow, indicate the possibility of variations in contamination sources or processing methods.

In Australia, a comprehensive survey revealed that generic *E. coli* was present in 2.3% of beef carcasses, 28.4% of sheep carcasses, and 5.4% of pork carcasses. These results reflect improvements in total bacterial loadings compared with previous national baseline surveys but still indicate substantial contamination levels (Jolley et al., 2023).

The study by Lee et al., conducted over a one-year period in Southern California, found an overall prevalence of *E. coli* in retail meat samples at 47.51%. Notably, poultry exhibited higher odds for resistance to multiple drugs compared to non-poultry meats. This suggests that poultry not only harbors more *E. coli* but also more resistant strains (K. Y. Lee et al., 2023) .

A comprehensive study (Hussein, 2007) assessed the prevalence rates of Shiga toxin-producing *E. coli (STEC)* in beef cattle and their products over three decades. The findings indicated that the prevalence rates of *E. coli O157* ranged from 0.1% to 54.2% in ground beef, with similar variability observed in other beef products such as sausages and retail cuts. Non-O157 STEC serotypes also showed significant prevalence rates, ranging from 2.4% to 58%, depending on the product type.

In Brazil, (G.M. Gonzalez & M.F. Cerqueira, 2020) reported that the prevalence of STEC in cattle reached up to 90%, with a corresponding prevalence rate of 20% in meat products. This high prevalence underscores the critical need for stringent control measures during meat processing to ensure food safety.

Similarly, a study conducted by (Nehoya et al., 2020) in Namibia found that 41.66% of raw beef samples from both informal and commercial abattoirs tested positive for STEC, with major strains characterized by virulence genes such as *eae, stx1*, and *stx2*.

The presence of virulent strains like O157:H7 has been documented even among wildlife reservoirs such as wild Scottish deer as shown by (Fitzgerald et al., 2023), indicating potential spillover risks into human populations through game meat consumption.

The results of the antibiotic susceptibility testing showed that *E. coli* exhibited a high sensitivity to cefoperazone (85.7%) and a moderate to high sensitivity to gentamycin, nitrofurantoin, ciprofloxacin, piperacillin/tazobactam, and cefoperazone/sulbactam. This suggests that these antibiotics could be effective in treating *E. coli* contamination in meat products. Significant resistance was detected for norfloxacin and amoxicillin/clavulanic acid, with resistance rates of 41.2% and 34.9% respectively.

research from Portugal indicated that broiler meat had the highest frequency of *E. coli* non-wild type to third-generation cephalosporins at 30.3% (Clemente et al., 2021). This finding aligns with global trends where poultry often shows higher contamination rates compared to other meats.

(Baah et al., 2022) conducted a cross-sectional study in Accra, Ghana, where they collected 270 meat samples (beef, goat meat, and chicken) and found that *E. coli* was the predominant contaminant (44.6%). They reported a moderate prevalence of multidrug-resistant (MDR) bacteria at 14.9%, with specific resistance patterns including amikacin, ampicillin, cefuroxime, ceftriaxone, ceftazidime, ciprofloxacin, trimethoprim-sulfamethoxazole, ertapenem, meropenem, imipenem, tigecycline, and gentamicin. This study highlights the significant presence of MDR *E. coli* in retail meats.

(Rega et al., 2022) examined pork and wild boar meat for AMR *E. coli* in Italy. They found quinolone resistance in 41.9% of pork-derived *E. coli* and aminoglycoside resistance in 6.6%. No carbapenem-resistant profiles were detected. This study indicates that while quinolone resistance is prevalent among pork isolates, aminoglycoside resistance remains relatively low.

Lastly, (Gugsa et al., 2022) research on bovine-origin foods in Ethiopia identified Shiga toxin-producing *EHEC O157:H7* with high multidrug-resistance rates at 75%, highlighting an urgent need for comprehensive phenotypic and molecular characterization efforts nationwide.

The study examines the antibacterial activity of different plant extracts, such as rosemary, artemisia, cinnamon, clove, and zaatar, against *E. coli* isolates found in meat products. The findings suggest that the reaction to the dosage

of plant extracts is directly proportional, meaning that higher concentrations of the extracts have a stronger inhibitory impact on the growth of *E. coli.*

Rosemary consistently exhibited the highest minimum inhibitory concentration (MIC) values across all tested concentrations, demonstrating its substantial potential as an antibacterial agent. Artemisia demonstrated a significant reduction in MIC values as its concentration was decreased from 100 mg to 25 mg, indicating a strong and dose-dependent antibacterial activity. Similarly, both cinnamon and zaatar exhibited potent antibacterial effects. Notably, cinnamon displayed lower minimum inhibitory concentration (MIC) values, indicating its strong effectiveness in suppressing the development of *E. coli*.

The antibacterial effect of plant extracts is likely caused by being able to disrupt bacterial cell membranes, inhibit major enzymes, or impede metabolic pathways. The results highlight the possible use of plant extracts as natural preservatives or antimicrobial substances in food products, providing a hopeful substitute for synthetic preservers and perhaps reducing the possibility of antibiotic resistance emergence.

(Hamad et al., 2020) evaluated the antibacterial activity of seven herbal plants and their combinations against *E. coli O157:H7* in meat and fish products. They found that clove aqueous extract exhibited the greatest inhibition zone (25 mm), while a combination mix (clove, sage, pomegranate, and Cassia fistula) showed the highest inhibition with a minimum inhibitory concentration (MIC) of 3.0 mg/mL.

(Meshaal et al., 2021) investigated the antimicrobial activity of essential oils and spices powder from cumin, black seeds, cloves, cinnamon, and marjoram against *E. coli O157:H7* isolated from raw and processed meat samples. Their results indicated significant reductions in microbial counts in treated samples compared to controls during storage periods. Notably, essential oils achieved zero microbial count after 15 days of storage, highlighting their potent antimicrobial properties.

Clove essential oil has demonstrated significant antimicrobial activity against *E. coli* in multiple studies. (Radünz et al., 2019) found that clove essential oil exhibited both inhibitory and bactericidal effects against *E. coli* among other pathogens.

Similarly, (Kong et al., 2007) reported that clove oil dissolved in ethanol effectively suppressed *E. coli* growth in culture media and vacuum-packaged pork, achieving microbial reductions between 1.81-2.32 log CFU/g over a storage period of up to 28 days.

The cinnamon extract also showed promising results. (Mahmud et al., 2023) utilized a mixture design methodology to optimize an antimicrobial formulation containing cinnamon essential oil among other components. This formulation was effective against *E. coli O157:H7* with minimal inhibitory concentrations (MICs) demonstrating potent antibacterial activity.

Rosemary extract's efficacy was highlighted by (Soyer et al., 2020), who found that combinations of activated lactoferrin and rosemary extract significantly inhibited *E. coli O157:H7* growth in meat samples through synergistic effects, reducing bacterial counts by approximately two logs.

(Hernández-Ochoa et al., 2014) investigation into cumin and clove essential oils revealed substantial reductions in pathogenic bacteria including *E. coli O157:H7* when applied to meat samples at varying concentrations. The study identified eugenol as the primary active compound responsible for the observed antimicrobial effects.

Our study also utilized PCR assays to identify enterotoxins and antimicrobial-resistant genes in E. coli isolates following exposure to plant extracts. The target genes, including *fliC, stx1, stx2, eae, and rfbE*, are recognized indicators of virulence and pathogenicity in *E. coli*.

The existence of the *fliC* gene indicates an increased ability to move and establish colonies, which contributes to the bacterium's potential to cause disease. The *stx1* and *stx2* genes, which encode Shiga toxins, play a crucial role in detecting strains that have the potential to cause serious diseases such as hemorrhagic colitis and hemolytic uremic syndrome (HUS). The *eae* gene is crucial for the formation of attachment and effacing lesions that are characteristic of enteropathogenic and enterohemorrhagic *E. coli* infections. The *rfbE* gene has a role in the production of O-antigens, which are crucial for bacterial variation and disease-causing ability.

(Sallam et al., 2013) provides valuable insights into this issue through a detailed examination of *E. coli* O157 strains isolated from retail beef products in Mansoura, Egypt.

The study reports contamination rates with *rfbEO157*-positive *E. coli* O157 strains at 26.7% in ground beef, 10% in beef burgers, and 3.7% in fresh beef samples, resulting in an overall mean contamination rate of 13.8% among all meat products tested. This high prevalence underscores the significant risk posed by these pathogens in retail meat products.

PCR assays confirmed the presence of *stx1* and *stx2* genes in a significant proportion of the isolates: *stx1* was detected in 46.7% (7/15), while *stx2* was found in 86.7% (13/15). Notably, two isolates were negative for Shiga toxin genes altogether, highlighting potential variability or loss of virulence factors within some strains.

The *eae* gene, essential for intimate attachment to host cells and formation of attaching and effacing lesions, was identified in nearly all isolates except one. Interestingly, a combination of four virulence genes—*stx1*, *stx2*, *eae*, and *EHEC*-hlyA—was detected in nearly half (46.7%) of the strains.

(G.M. Gonzalez & M.F. Cerqueira, 2020) reported that in Brazil, the prevalence of STEC reaches up to 20% in meat products, with *stx2* being more frequent than *stx1*. This finding aligns with other studies indicating a higher pathogenic potential associated with *stx2*-positive strains. In a study by (Haque et al., 2022), the prevalence rate of six-positive *E. coli* isolates in retail pork samples varied significantly depending on geographical location and hygiene conditions, ranging from 0.10% to 80%. This wide range underscores the variability influenced by local practices and detection methods.

(Tutija et al., 2022) characterized *E. coli* isolates from calves in Brazil, finding high frequencies for both *stx1* (76.70%) and *stx2* (35.22%). The majority were multidrug-resistant, complicating treatment options for infections caused by these pathogens.

(Nehoya et al., 2020) assessed raw beef samples from abattoirs in Namibia; they found an overall STEC prevalence rate of 41.66%, with significant proportions harboring major virulence genes like *eae* and both Shiga toxins.

(Menck-Costa et al., 2023) focused on commercial meat samples across different types (chicken, pork, beef) revealing substantial resistance profiles along with virulence gene presence including *stx1* and *stx2*.

Our study found that heat and UV radiation had varying effects on the identification of virulence genes in *E. coli*. Following the application of heat treatment, the presence of the *fliC* and *stx-1* genes was not detected, however, the *eae* and *rfbE* genes remained observable. UV exposure led to the non-detection of the *fliC* and *stx-1* genes, but the *eae* and *rfbE* genes were present .

The results indicate that the *fliC* and *stx-1* genes are more vulnerable to the impacts of heat and UV radiation, either because of DNA damage or changes in gene expression. In contrast, the *eae* and *rfbE* genes demonstrated resilience to various stressors, suggesting their possible contribution to the survival and endurance of *E. coli* in unfavorable circumstances.

The observed variations in gene expression in response to heat and UV radiation could have important consequences for the development of efficient decontamination techniques and the comprehension of bacterial adaption processes. Additional investigation is required to clarify the fundamental molecular processes and examine the possible consequences for the harmfulness and strength of *E. coli* in different environmental circumstances.

(Machado et al., 2023) conducted a study on *Escherichia coli* isolates from Brazilian beef, focusing on heat resistance profiles and the presence of transmissible locus of stress tolerance (tLST). They found that while some *E. coli* isolates exhibited significant heat resistance, *stx* genes were absent in tLST-positive isolates. This suggests that heat-resistant strains may not necessarily carry *stx1* or other Shiga toxin genes, complicating the direct correlation between heat resistance and virulence gene presence.

(Zhang et al., 2020) developed a multiplex PCR assay for identifying diarrheagenic *E. coli* (DEC) pathotypes based on nine virulence genes, including *stx1* and *stx2*. Their method showed high specificity and sensitivity, making it a robust tool for detecting virulence genes even after environmental stress treatments such as heat or UV exposure.

(Mageiros et al., 2021) provided insights into genome evolution and pathogenicity emergence in avian pathogenic *E. coli* (APEC). They identified genetic elements associated with pathogenicity that include responses to environmental stresses like heat shock proteins and antimicrobial resistance genes. These findings underscore the complexity of virulence gene regulation under different environmental conditions.

## 4. Conclusion

This study reveals significant insights into the frequency, resistance patterns to antibiotics, and the impact of plant extracts, heat, and UV radiation on the expression of virulence genes in *E. coli* obtained from red meat samples. The results emphasize the significance of developing efficient measures to ensure food safety, using antibiotics carefully, and investigating natural antimicrobial substances as possible alternatives to fight against foodborne infections and reduce the probability of antibiotic resistance. Furthermore, this investigation enhances our comprehension of the intricate relationship between environmental conditions, gene

expression, and bacterial adaptability mechanisms. This progress provides up opportunities for additional research and the creation of focused approaches to control pathogens and ensure food safety.

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