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# Isolation of Antibiotic-Resistant Pathogenic Bacteria from Traditionally Slaughtered Chickens in Khartoum State

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**Abstract:** This study was conducted to investigate the presence of microbial contaminants, with a focus on certain pathogenic and antibiotic-resistant bacteria, in the meat of free-range chickens slaughtered using traditional methods in Khartoum State.

The study involved collecting 30 samples of chicken meat from three different markets, with the samples divided into internal and external samples. The results led to the isolation of six bacterial genera, namely: Escherichia coli, , Staphylococcus aureus, Salmonella spp., Pseudomonas spp., Proteus spp., and Bacillus spp. The results showed that the highest bacterial count was recorded in the May Market (Market 6), reaching $1.05 \times 10^5$ CFU/g, compared to the Al-Ashar Market in Khartoum, where the count was $8.51 \times 10^4$ CFU/g, and the Omdurman Market, where it reached ($9.33 \times 10^4$ cells/g). These results indicate a high level of bacterial contamination in traditionally slaughtered chicken meat, with clear variation among the different markets. Regarding the antibiotics under study, the results showed that Escherichia coli isolates exhibited the highest resistance rate of 100% to Ampicillin, while their resistance rate to Tetracycline was approximately 65.5%. Salmonella spp. isolates also showed complete (100%) resistance to Ampicillin. Pseudomonas spp. and Proteus spp. isolates, however, exhibited the highest levels of resistance, with a resistance rate of 100% for each of the following antibiotics: Ampicillin, Cefotaxime, Chloramphenicol, Ciprofloxacin, and Levofloxacin. These results reflect a notable prevalence of multidrug-resistant bacterial strains, posing a health risk that warrants further monitoring and control. The results showed that the antibiotics cefracycline and ofloxacin were the most effective against the bacterial isolates under study, with a 100% efficacy rate, while the isolates exhibited varying resistance to the other antibiotics. Molecular typing of Escherichia coli and Salmonella spp. isolates was also investigated, and the diagnosis was confirmed using molecular markers specific to each species, with the 16S rRNA gene used for E. coli and the FliC-d gene used for Salmonella. Specific primers were designed for each gene, and agarose gel electrophoresis results showed that all isolates possessed the target genes, with some base changes in certain genes compared to the reference sequences. Thus, the molecular diagnosis of both E. coli and Salmonella was confirmed using the 16S rRNA and FliCd genes, respectively.

**Keywords:** Antibiotic-resistant bacteria, Molecular typing, Khartoum, PCR

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## INTRODUCTION

Meat is a staple food and is found in almost every meal, whether breakfast, lunch, or dinner (Al-Nazzal, 2015). Poultry meat consumption has increased significantly over the past two decades, as it is considered a healthy alternative to red meat, The increase in the consumption of poultry meat and its products has been accompanied by the spread of diseases caused by certain pathogenic bacteria, especially since broiler chickens and other types of poultry are among the foodstuffs most contaminated with disease-causing microorganisms (Khalid, 2016). Poultry is considered one of the most important sources of animal protein for humans worldwide, in the form of meat and eggs. Raising chickens at home is a widespread activity in most areas of Khartoum State, providing an important source of animal protein and additional income for lowincome families; however, these practices are often carried out under unsanitary conditions and using primitive traditional methods of rearing, treatment, and slaughter, creating an environment conducive to the proliferation of pathogenic bacteria transmissible to humans (Marchall and Levy 2011). Food contamination is a serious problem, as approximately 20% of the world's population is at risk due to contamination of a wide range of foods, including meat and meat products (Abdulkarim et al., 2024). The microbial quality of meat is determined by the number and type of microorganisms growing in it. Meat is not free of microorganisms, but their numbers may increase if conditions suitable for their growth—such as

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temperature, humidity, and oxygen—are present (Ashour et al., 2021). Bacterial resistance to antibiotics has become one of the greatest challenges facing global public health (WHO, 2017). Bacterial infections constitute a global health issue, particularly antibiotic-resistant infections ( ). The emergence of multi-drug-resistant bacterial strains and the shortage of antibiotics are a cause for concern for all of humanity (Al-Hata, 2021). In Sudan, particularly in Khartoum State, backyard chicken farming and slaughter using traditional methods are widespread, without veterinary supervision or adherence to hygiene standards, which increases the likelihood of pathogenic bacteria such as *Escherichia coli, Salmonella spp., S. aureus, Pseudomonas* aeruginosa (Daka et al., 2018). Therefore, bacteria—whether pathogenic or non-pathogenic—may generally be present on raw or undercooked chicken, just like any perishable food. and the danger of these microorganisms lies in their ability to transmit genes conferring resistance to multiple antibiotics, which limits the effectiveness of medical treatments and poses a direct threat to public health (Bogaerts *et al*. 2019). Over the decades, researchers' efforts have focused on advancing human health and improving the quality of life in communities, recognizing that public health is closely linked to the availability of safe, wholesome food free from pathogenic microorganisms, as clean food is the cornerstone of preventing foodborne diseases (Melhem, 2016).Given the scarcity of studies and information regarding the sanitary conditions of traditional local chicken slaughterhouses and their impact on meat quality, consumer safety, and the environment, it became necessary to assess the current sanitary status of some of these slaughterhouses located within the three districts of Khartoum State. This was done by conducting health inspections on samples taken from these slaughterhouses to determine their level of compliance with approved health requirements. The establishment of modern slaughterhouses has contributed to the development of this industry, as it has helped address many of the shortcomings that traditional slaughterhouses suffered from, such as carcass contamination, limited production capacity, poor efficiency of cooling and freezing systems, and the suboptimal utilization of byproducts (Faraj, 2024). Accordingly, this study aims to assess the level of bacterial contamination in carcass samples, while identifying some of the pathogenic bacterial species associated with them. The study also seeks to isolate and determine the molecular classification of certain pathogenic bacteria resistant to a number of antibiotics, and to study their resistance patterns, given the importance of this in understanding the nature of resistance spread in the local environment, assessing the extent to which traditional local chicken slaughterhouses comply with applicable health standards and specifications, and evaluating the risks of transmission to humans.

In light of the expected results, the study will provide scientific recommendations to help limit the spread of antibiotic-resistant bacteria and improve hygiene practices during chicken rearing and slaughter, thereby enhancing food safety and protecting public health.

## The Enterobacteriaceae family

Bacteria belonging to this family are characterized by being Gramnegative rods. They are motile via flagella or non-motile and non-sporeforming. They grow in standard agricultural media; all ferment glucose with or without gas production. They are facultative anaerobes; many are intestinal pathogens or commensals, while a few are saprophytic. They are also found in soil and water, possess complex metabolic pathways, and produce various types of toxins and virulence factors (Al-Otaibi, 2013). This family includes a number of bacterial genera such as *Escherichia, Shigella, Salmonella, Enterobacter, Klebsiella, Serratia, and Proteus.* Some of these organisms, such as *Escherichia coli,* are naturally present in the intestine but can sometimes cause disease in humans, while others, such as Salmonella and *Shigella*, are typically pathogenic to humans (Hamad al-Nil and alHassan, 1999).

## Colon Bacteria: Escherichia coli

This bacterium was first classified by the scientist Theodor Escherich in 1885 in Germany and was then named *Bacterium ecoli*; it is now known as *Escherichia coli,* as it was isolated from the feces of healthy children ( ) and was therefore considered non-pathogenic at the time (Cookes, 1985).This bacterium is characterized by being small rods (0.5–1.3 µm) that are Gramnegative and motile via peritrichous flagella. Some of its strains may have a non-spore-forming, facultatively anaerobic or aerobic phenotype, with an optimal growth temperature of 37°C،but they can grow over a wide temperature range of 15–45 $^{\circ C}$(Al-Otaibi, 2013). They ferment glucose and lactose, producing acids and gases ($Co_2$-$H_2$) in equal amounts; the neutral compound is not acetylmethylcarbinol, which is positive with methyl red. It does not use uric acid as a nitrogen source and produces indole (Shuaib and Eissa, 2014). It grows on differential agricultural media in the presence of (EMB) (Eosin Methylene Blue) medium, which produces a shiny metallic sheen. *E. coli strains* may be pink or red with a purplish tint. These bacteria produce colors on food as they grow (Al-Rajab and Al-Ghazaz, 1982).It is found in the human large intestine and can be isolated from feces; it causes disease in humans when present in very large numbers (Shuaib and Issa, 2014). Diseases caused by this bacterium include urinary tract infections and meningitis, and it infects soft tissues; therefore, it is considered an opportunistic bacterium (Al-Otaibi, 2013).

### Genus *Enterobacter*

Rods ranging in length from 1.2 to 3 micrometers. Gram-negative, motile via 4–6 peritrichous flagella. Capable of fermenting glucose, producing $CO_2$and $H_{22}$ in a 1:2 ratio; however, at 44.5°C, no gas is produced during glucose fermentation. Most strains test positive for the FugisProskauer test (production of ethyl methyl carbinol), negative for the methyl red test, and have a weak ability to liquefy gelatin (Abu Al-Dahab et al., 2011). The bacteria grow on MacConkey medium, forming pink, mucoid colonies that are less viscous (mucous) than in *Klebsiella* bacteria. The bacteria ferment mannitol and produce gas from the fermentation of certain sugars. These bacteria are found in the human gastrointestinal tract, soil, and water (Al-Otaibi, 2013).

### The genus Staphylococcus

Scientist Alexander Ogston was the first to describe Staphylococcus in 1883, naming it *Staphylococci*, a term derived from the Greek words Staphyle, meaning “bunch of grapes,” and Coccos, meaning “wheat grains” (Al-Tamimi, 2012). They consist of non-motile, Gram-positive spherical cells. Some species are yellow or orange in color and insoluble in water, particularly in environments containing relatively high concentrations of table salt. They decompose arginine, producing ammonia gas, They also reduce nitrates and are capable of fermenting various carbohydrates; they are positive for the catalase test. Strains capable of lysing red blood cells can secrete toxins into the host’s body or growth environment, including *Staphylococcus aureus* (Shuaib & Issa, 2014).Staphylococci are divided into two groups based on their production of coagulase. Coagulase-producing staphylococci include the most important pathogen for humans, *Staphylococcus aureus* (Al-Tamimi, 2012).

### Staphylococcus aureus

*Staphylococcus aureus* is one of the most important species within the genus Staphylococcus and is distinguished from others by its production of the coagulase enzyme. This species also possesses several important cell wall components and antigens (Hamad al-Nil and al-Hassan 1999). However, there are rare strains that may be negative for this enzyme, although some species that cause diseases in other animals, such as Staphylococcus intermedia and Staphylococcus hyicus, are positive for the coagulase enzyme (Al-Tamimi, 2012). This bacterium is present in the human body as part of the normal microflora (normal microflora), with the most prominent sites being the skin, nasal cavity, nasopharynx, armpits, groin, anal region, genital region, and rectum; they can colonize various epithelial or mucosal surfaces (Al-Tamimi, 2012).

### The genus *Proteus*

The scientist Hauser was the first to discover Proteus bacteria in 1885 when he first isolated them from sewage, decomposing organic matter, and feces, and named them Proteus due to their pleomorphism (Al-Otaibi, 2013). The genus *Proteus consists* of short, Gram-negative rods approximately 1–3 microns in length and 0.8–4 microns in width. They are characterized by pleomorphism, appearing in various forms, including short, spherical rods that are typically non-motile, or long, filamentous forms characterized by active movement. They possess peritrichous flagella that aid in rapid movement, and this movement is clearly visible on the surface of solid culture media in the form of concentric circles. This movement occurs in waves, a phenomenon known as swarming, which resembles swimming (Al-Otaibi, 2013).

### The genus Salmonella

Motile bacilli with peritrichous flagella that do not produce indole, produce hydrogen sulfide, or are variable test (producing acids only upon fermentation of sugars other than lactose and sucrose, i.e., disaccharides) (Abu Al-Dahab et al., 2011).Salmonella vary in length; most are motile via flagella distributed across the cell surface and can survive in frozen water for long periods (Hamad Al-Nile and Al-Hassan, 1999). It belongs to the Enterobacteriaceae family and is widespread in nature. These bacteria are found in the digestive tracts of mammals, birds, and reptiles, as well as in sick and convalescent individuals. There are a great many serotypes of Salmonella; all serotypes cause infections, but the most medically significant serotypes are:

- Species that infect humans only: Salmonella typhi *(S. typhi)*, Salmonella paratyphi *A (S. paratyphi A),* and Salmonella paratyphi *B (S. paratyphi B)*. These serotypes cause typhoid fever in humans only.
- Types transmitted from animals to humans via the digestive tract: Salmonella enterica *S. enteridis* and Salmonella typhimurium *S. typhimurium* cause food poisoning or enterocolitis in humans and primarily infect animals and birds; they are likely to be present in any fresh poultry product, especially chicken eggs and fresh chicken meat. (Al-Bashara, 2014).

### The genus *Pseudomonas spp.*

This genus comprises more than 200 bacterial species. It causes opportunistic infections in hospitals and in immunocompromised individuals; the most pathogenic species for humans is Pseudomonas *aeruginosa* (Al-Bashara, 2014). It is a Gram-negative, rod-shaped bacterium that moves using polar flagella; most species secrete water-soluble pigments into the external environment (Yasri, 2005).

## Materials and Methods

### *Sample Collection*

Thirty samples were collected from locally raised free-range chickens slaughtered traditionally in

Khartoum State. Fifteen chickens aged 6–8 weeks were purchased randomly from vendors in three different areas of Khartoum State, namely (Mayo Market 6, Al-Asharah Popular Market in Khartoum, and Omdurman Market). The samples were collected between December 25, 2016 ( ) and February 18, 2017. The samples included (parts taken from various chicken meat, namely the breast, back, legs, wings, and internal organs). The chickens were slaughtered and cleaned at the vendors' shops, where slaughtering is carried out without regard for regulations. The method of collecting samples to detect the presence of pathogenic bacteria in locally slaughtered chicken can be summarized as follows:

- The sample was taken using the same tools used by the vendor
- The samples were placed in pre-sterilized sample collection bags equipped with a sealing strip and labeled with an identification tag bearing the sample number, name, and collection date; these bags were transported to the laboratory under refrigerated conditions
- The samples were tested within 24 hours

### Culturing the samples

Samples were cultured from the chicken's internal organs, including the heart, gizzard, and intestines, as well as from external parts such as the breast, legs, and back. The samples were transported immediately after collection under sterile conditions to the laboratory, where they were inoculated onto culture media suitable for the growth of the bacteria under study.

To isolate and culture Salmonella, the samples were selectively inoculated onto one of the following selective media:

***Salmonella***

Bismuth Sulfite Agar
Xylose Lysine Deoxycholate Agar

For the isolation and culture of Escherichia coli, the following differential media were used:

***Escherichia coli***

Eosin Methylene Blue Agar
MacConkey Agar

This was done to obtain pure isolates in preparation for subsequent morphological, biochemical, and molecular tests.

Methods for isolating bacteria belonging to the *Enterobacteriaceae* family

First: Bacteria belonging to the *Enterobacteriaceae* family ( ) were isolated and identified according to Ranjan (2007) and Morello *et al*. (2006):

1. 25 g of chicken meat was added to 225 mL of physiological saline (composed of 8 g sodium chloride and 1 g peptone; the volume was made up to 1000 mL with distilled water and mixed thoroughly), thereby obtaining the first dilution. The procedure was performed in a sterile environment.
2. One milliliter of the first dilution was pipetted and added to a tube containing 9 mL of physiological dilution solution to obtain the second dilution. The process was then repeated to reach the desired dilution
3. Transfer 1 mL of the desired dilution under sterile conditions to a sterile Petri dish; use two replicates for each dilution.
4. Sterile MacConkey agar medium was added to the plates. The solution was then mixed with the medium by swirling the plate in a circular motion, first clockwise and then counterclockwise, and the plates were left to solidify. The plates were then incubated upside down in an incubator at 37°C and 44.5°C for 48 hours, with two replicates per temperature.
5. The pink-colored, growing colonies on MacConkey agar at both incubation temperatures were considered typical colonies. They were transferred to methylene blue Eosin (EMB) medium using the streaking method in a sterile atmosphere, and the plates were incubated upside down in an incubator at 37°C for 24 hours.
6. A series of biochemical tests were performed on the growing isolates, such as Gram staining and microscopic examination, in addition to another set of biochemical tests:

### Bacterial identification by fermentation of various sugars.

### Biochemical tests

### A. Catalase Test

This test was performed to determine the bacteria's ability to grow in the presence of hydrogen peroxide $(H_2O_2)_2$. Three milliliters of the ($H_2O_2$ 3%) were placed in a test tube, and a swab from a single colony of a pure culture was added using a sterile glass rod and inoculated into the tube. Bubble formation is considered indicative of a positive test, while the absence of bubble formation indicates a negative result (Cheesbrough, 1985)

### B. Oxidase Test

This test is used to determine the ability of bacteria to produce cytochrome oxidase and, consequently, the ability to reduce oxygen. A filter paper is moistened with the oxidase reagent (Titramethyl Paraphenylene Diamine Dihydrochloride), and a smear from the colony is transferred to that paper. The formation of a pink color indicates a positive test result. Enterobacteriaceae bacteria are considered oxidase-negative, as there is no change in the color of the filter paper containing the reagent (Hemraj et al., 2013)

### C. Indole Test

This test was used to detect the presence of indole, which is a metabolite of the amino acid tryptophan resulting from the bacteria's possession of the enzyme tryptophanase. The bacteria were inoculated into liquid peptone broth and the medium was incubated at 37°C$^5$ for 24 hours, then five drops of Kovac's reagent were added to the inner surface of the test tube; the appearance of a red ring indicates a positive result (Koneman et al. 1992).

### D. Methyl Red Test

This test was conducted to distinguish between bacteria based on their fermentation of glucose and the production of acids in the environment. Many Gram-negative bacteria living in the human intestine can ferment glucose, producing large amounts of lactic acid, acetic acid, succinic acid, and formic acid, in addition to $CO_2$, alcohol, and hydrogen. The accumulation of these acids in the environment lowers the pH; if methyl red indicator is added to the culture, a red color will appear, indicating the production of these acids (Akhtar et al., 2001). This was carried out according to the following steps (Jaswinder et al., 2004), (Cheesbrough, 1985)

- A tube containing MR-VP medium was inoculated with a fresh culture
- It was incubated at 37°C for 48 hours
- The bacteria's ability to produce acid was tested by adding several drops of methyl red indicator to the culture (it turns red in an acidic medium and yellow in a basic medium); the appearance of red indicates a positive test.

### E. Urease Test

This test demonstrates the bacteria's ability to produce urease and, consequently, to break down urea and produce ammonia. A portion of the colony was transferred using a sterile needle to a slanted urea agar medium and incubated at 37°C ( ) for 24 hours.If the bacteria produce urease, the urea breaks down into ammonia and the medium turns pinkish-red (Cheesbrough, 1985).

### Citrate Test

This test determines the bacteria's ability to use citrate as the sole carbon source. The plate containing the citrate agar medium is inoculated with fresh colonies and incubated for 24 hours at 37°C. No change in the color of the medium indicates a negative test, while a change in color from green to blue indicates a positive test. Bacteria capable of using citrate as a carbon source grow in this medium (Hemraj et al., 2013).

### K. Kligler iron agar test

The tubes containing KIA medium are inoculated by streaking the isolates to be diagnosed onto the slant and incubated at 37°C$^5$ for 24 hours. The result is based on changes in pH, and fermentation can be observed by the indicator turning from red to yellow. http://www.bd.com//ds.clincol.

### Classification Based on Biochemical Reactions

The data in Table (1) show the characteristics of some of the studied bacterial species based on certain biochemical tests regarding carbohydrate fermentation, blood sugar analysis, and indole ring formation Table 1: Results of the classification of Gram-negative and Gram-positive isolates based on biochemical tests.

**The data in Table 1: Classification Based on Biochemical Reactions**

| | Catalase | Oxidase | Lactose fermentation | Glucose fermentation | Maltose fermentation | Hemolysis | Indole | Urea |
|---|---|---|---|---|---|---|---|---|
| *E. coli* | + | - | + | + | + | +\- | + | - |
| *Salmonella* | - | - | - | + | + | - | - | - |
| *Pasteurella* | + | + | - | + | - | - | - | - |
| *Pseudomonas* | + | + | - | - | - | + | - | - |
| *Staphylococcus* | + | - | + | + | + | +\- | - | - |

### Estimation of the total number of aerobic bacteria (Total Plate Count)

For this test, use Plate Count Agar medium. Transfer 1 mL of the dilution to a Petri dish using a micropipette, then pour it into the medium after cooling it to 45°C. Gently swirl the dishes to ensure good mixing and distribution, allow them to solidify, then invert the dishes and incubate at 37°Cfor 24 hours. Afterward, count the number of bacteria growing in the plates.

**Antibiotic Susceptibility Test**:

The standard Bauer-Kerby method was used to test bacterial susceptibility to antibiotics (Vandepitte et al. 1991).

1. 5 mL of peptone water was inoculated with 2–3 colonies from 24-hour-old pure bacterial cultures.
2. The tubes were shaken well and incubated in an incubator at$^{37^{\circ}C}$for two hours.
3. 0.1 mL of the bacterial suspension was transferred and spread onto MuellerHenton agar medium. The plate was left at room temperature for 5 minutes until the culture dried.
4. Antibiotic discs were transferred to the surface of the culture medium using sterile forceps at a rate of 5 discs per plate.
5. The plates were incubated at$^{37^{\circ}C}$for 24 hours, and the diameters of the inhibition zones around each disc were measured and recorded by measuring the

diameter of the inhibition zone around each disc with a micrometer (ruler) and then compared to the international measurement system. Bacteria were classified as sensitive "S," resistant "R," or intermediate "I" according to the standard specifications provided by NCCLS (2007) using sterile forceps (concentrated alcohol, flame sterilization), with 5 discs per plate, ensuring an appropriate distance of 15 mm between each antibiotic to avoid overlap between inhibition zones.

**DNA extraction according to the method of Altschul et al., 1990**

Extract DNA using the heat-and-cool method. Transfer 1 mL of the 24-hour-old bacterial culture to Eppendorf tubes.

Centrifuge the tubes at high speed (6000 rpm for 10 minutes), then discard the supernatant.

The cell pellet was resuspended by adding 10 µL of distilled water, and the tubes were shaken well. The tubes were then placed in a water bath at 95°C for 30 minutes. Afterward, they were cooled on ice for 10 minutes and centrifuged at 6000 rpm for 10 minutes; from this step, the supernatant—the extracted DNA—was collected.

### 3-5-1 Diagnosis Using Polymerase Chain Reaction (PCR):

The method of bacterial diagnosis using PCR is one of the most important methods because the DNA extracted from bacterial cells is amplified within one to two hours, making this method one of the fastest compared to traditional methods.

**Table 2: Bacteria were diagnosed using PCR with the 16S rRNA gene primer to diagnose *E. coli***

| **Primer** | Primer | Sequence | Length of the leader |
|---|---|---|---|
| 16 SRNA | Forward | 5' AGA GTT TGA TCA TGG CTC AG-3' | 20 bp |
| | Reverse | 5' GGA CTA CCA GGG TAT CTA AT-3' | 20 bp |
| Flic-d gene | Forward | 5-ACT CAG GCT TCC CGT AAC GC-3' | 20 bp |
| | Reverse | 5'- GGC TAG TAT TGT CCT TAT CGG-3' | 21 bp |

The reaction mixture is prepared as shown in Tables 1–2, with the forward primer, reverse primer, DNA, and deionized water added to the wells in several ACCUPOWER®PCR Premix containers containing the following components:

**Table 3: Reaction mixture for 16S rRNA Flic-d gene genetic diagnosis**

| **Components** | **Volume in µL** |
|---|---|
| Forward primer | 1 |
| Back primer | 1 |
| DNA | 1 |
| Master Mix | 4 |
| Distilled water | 18 |
| Final volume | 25 |

DNTps – $MgCl_2$– Taq – Maxime – PCR – 10× buffer

**Table 4: Optimal conditions for the detection of the 16 SRNA gene, for which the PCR machine was programmed.**

| **Step** | **Procedure** |
|---|---|
| (1) | One cycle (4 minutes) at 94°C for the first denaturation of the DNA template. |
| (2) | 36 cycles including<br>A- 60 seconds at 94°C to denature the template DNA.<br>B- 60 seconds at 56°C for primer annealing to the DNA template.<br>C- 60 seconds at 72°C for extension of the annealed primers. |
| (3) | One 7-minute cycle at 72°C for final extension of the amplified DNA strand. |

**Table 5: Optimal conditions for the detection of the Flic-d gene, for which the PCR machine was programmed.**

| **Step** | **Procedure** |
|---|---|
| (1) | One cycle for 4 minutes at 95°C. |
| (2) | 35 cycles consisting of<br>A- 60 seconds at 93°C for denaturation of the template DNA.<br>B- 60 seconds at 58°C for primer annealing to the DNA template.<br>C- 60 seconds at 72°C for extension of the annealed primers. |
| (3) | One 7-minute cycle at 72°C for the final extension of the amplified DNA strand. |

### Gel Electrophoresis

Gel electrophoresis of the extracted DNA was performed as described by Russell (2001) and Sambrook.

A 0.8% agarose gel was prepared by dissolving 0.8 g of agarose in 100 mL of 10x TBE solution after diluting it 10-fold to obtain 1x TBE. The agarose was heated to boiling and allowed to cool to a temperature of 45°C, then ethidium bromide dye was added at a concentration of 0.5 µg/mL. Using the stock solution prepared for this dye, the agarose was then mixed thoroughly.

A tray was prepared to hold the agarose, and the comb was secured to form the wells intended for sample loading. The agarose was then poured gently and continuously to avoid air bubbles, after which the gel was left to solidify at room temperature.

The comb was gently removed, and the gel, along with the mold, was transferred to a migration tank of suitable volume containing 1x TBE. The samples prepared for migration were loaded using a micropipette. The electrophoresis ( ) was then performed at a voltage of 75 V/ cm for 45 minutes until the samples reached just before the end of the gel. After completion of the electrophoresis, the mold was transferred for gel inspection by exposing it to a UV transilluminator at a wavelength of 340 nm, then photographed with a camera.

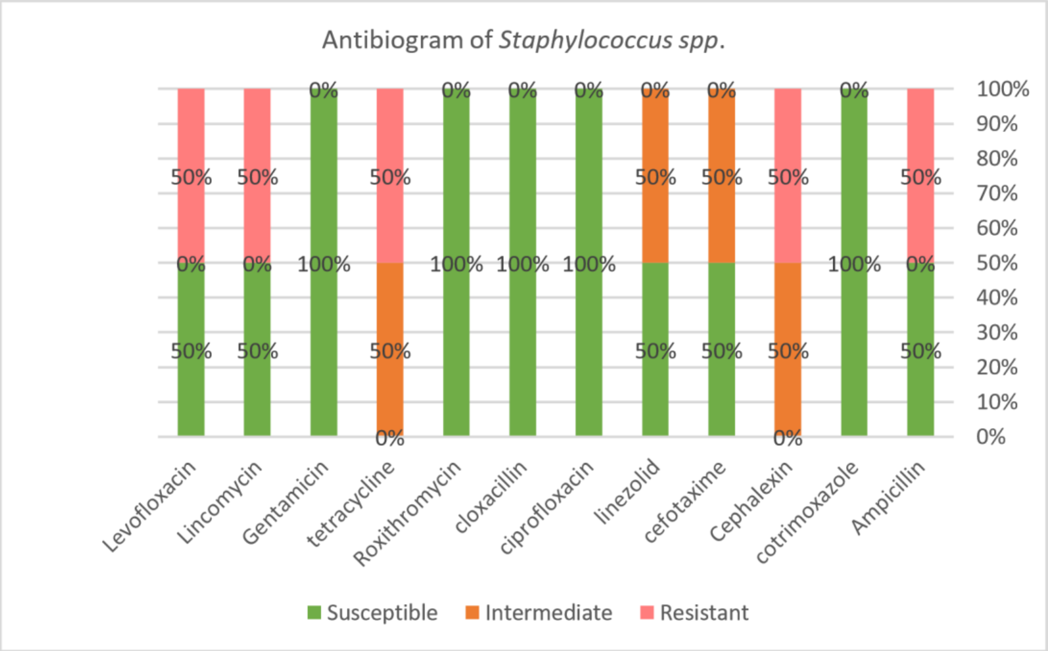
## RESULTS AND DISCUSSION

Table (6) shows the percentages of the presence of certain types of bacteria in samples of chicken meat sold in some markets in the state of Khartoum (May 6 Market, Al-Ashra Popular Market in Khartoum, and Omdurman Market)

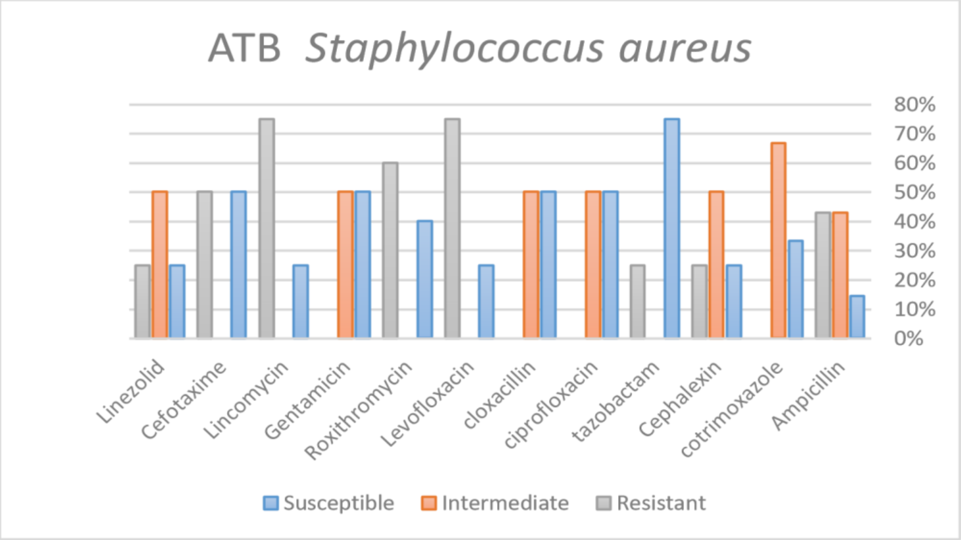
**Table 6**

| Number | Type of bacteria | Number of samples containing | Number of samples without bacteria | Percentage of samples containing bacteria |
|---|---|---|---|---|
| 1 | *E. coli* | 27 | 3 | 90% |
| 2 | *S. aureus* | 29 | 1 | 96.7% |
| 3 | *Salmonella spp* | 17 | 13 | 56.7% |
| 4 | *Proteus spp* | 7 | 23 | 23.3% |
| 5 | *Pseudomonas spp* | 12 | 18 | 40% |
| 6 | *Bacillus* | 1 | 29 | 3% |

Number of samples = 30



The results shown in this figure indicate that most isolates were susceptible, although some were 50% resistant and 50% sensitivity



From the figure, it can be seen that some of them were resistant to certain antibiotics

**Table 7: shows the ATB susceptibility testing of E. coli**

| ATB | Sensitive | Intermediate | Resistant |
|---|---|---|---|
| Ampicillin | 38% | 25% | 38% |
| Cotrimoxazole | 100% | 0% | 0% |
| Cefotaxime | 63% | 38% | 0% |
| Tazobactam | 75% | 25% | 0% |
| Chloramphenicol | 100% | 0% | 0% |
| Ciprofloxacin | 100% | 0% | 0% |
| Ceftriaxone | 100% | 0% | 0% |
| Tetracycline | 38% | 0% | 63% |
| Ofloxacin | 100% | 0% | 0% |
| Gentamicin | 100% | 0% | 0% |
| Amikacin | 100% | 0% | 0% |
| Levofloxacin | 100% | 0% | 0% |

The results of the antibiotic susceptibility testing of *E. coli* isolates showed complete susceptibility to most antibiotics; however, some isolates exhibited resistance to ampicillin and tetracycline at rates of 38% and 63%, respectively. The average susceptibility rates for ampicillin, tazobactam, and cefotaxime were 25%, 25%, 38%, respectively.

**Table 8: Shows the susceptibility testing of *Proteus spp***

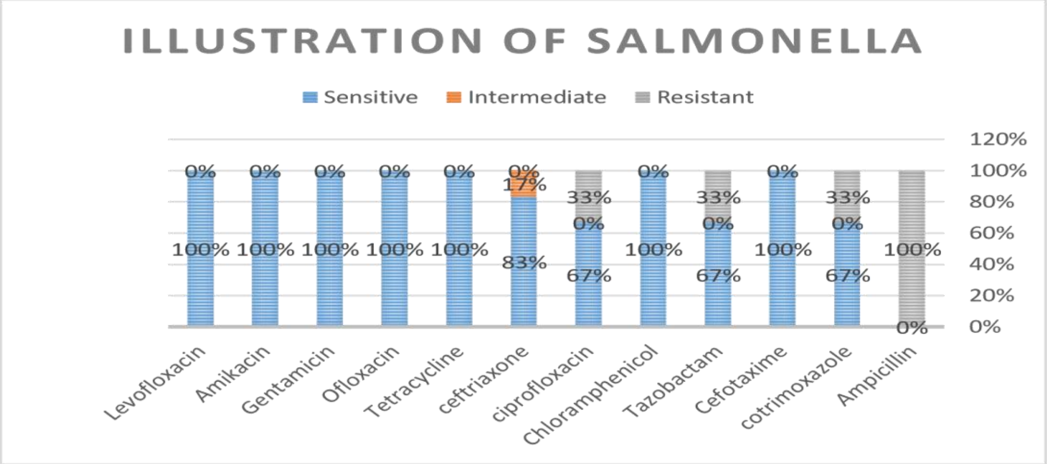
| ATB | Sensitive | Intermediate | Resistant |
|---|---|---|---|
| Ampicillin | 0% | 0% | 100% |
| Cotrimoxazole | 100% | 0% | 0% |
| Cefotaxime | 0% | 0% | 100% |
| Tazobactam | 50% | 50% | 0% |
| Chloramphenicol | 0% | 0% | 100% |
| Ciprofloxacin | 100% | 0% | 0% |
| Ceftriaxone | 100% | 0% | 0% |
| Tetracycline | 100% | 0% | 0% |
| Ofloxacin | 100% | 0% | 0% |
| Gentamicin | 100% | 0% | 0% |
| Amikacin | 100% | 0% | 0% |
| Levofloxacin | 0% | 0% | 100% |

The results of the sensitivity test for *Proteus spp.* to antibiotics showed complete resistance to four antibiotics, namely ampicillin, cefotaxime, chloramphenicol, and levofloxacin at a rate of 100%. Sensitivity was moderate for one antibiotic, tazobactam, at a rate of 50%, and the bacteria were sensitive to the remaining antibiotics.

**Table 9: Shows the antibiotic susceptibility testing of *Pseudomonas spp.***

| ATB | Sensitive | Intermediate | Resistant |
|---|---|---|---|
| Ampicillin | 0% | 0% | 100% |
| Cotrimoxazole | 0% | 0% | 100% |
| Cephalexin | 100% | 0% | 0% |
| Ceftriaxone | 0% | 0% | 100% |
| Tazobactam | 100% | 0% | 0% |
| Chloramphenicol | 0% | 0% | 100% |
| Ciprofloxacin | 50% | 50% | 0% |
| Tetracycline | 50% | 0% | 50% |
| Ofloxacin | 100% | 0% | 0% |
| Gentamicin | 100% | 0% | 0% |
| Amikacin | 100% | 0% | 0% |
| Levofloxacin | 100% | 0% | 0% |

The results in the table show that *Pseudomonas spp.* bacteria were resistant to four antibiotics, namely ampicillin, cotrimoxazole, ceftriaxone, and chloramphenicol at a rate of 100%, as well as 50% resistance to tetracycline and an average sensitivity of 50% to ciprofloxacin.



From this figure, we observe resistance to the following antibiotics: Ampicillin at 100%, cotrimoxazole at 33%, tazobactam at 33%, ciprofloxacin at 33%, and ceftriaxone at 17%. The remaining antibiotics showed sensitivity

**PCR Result**

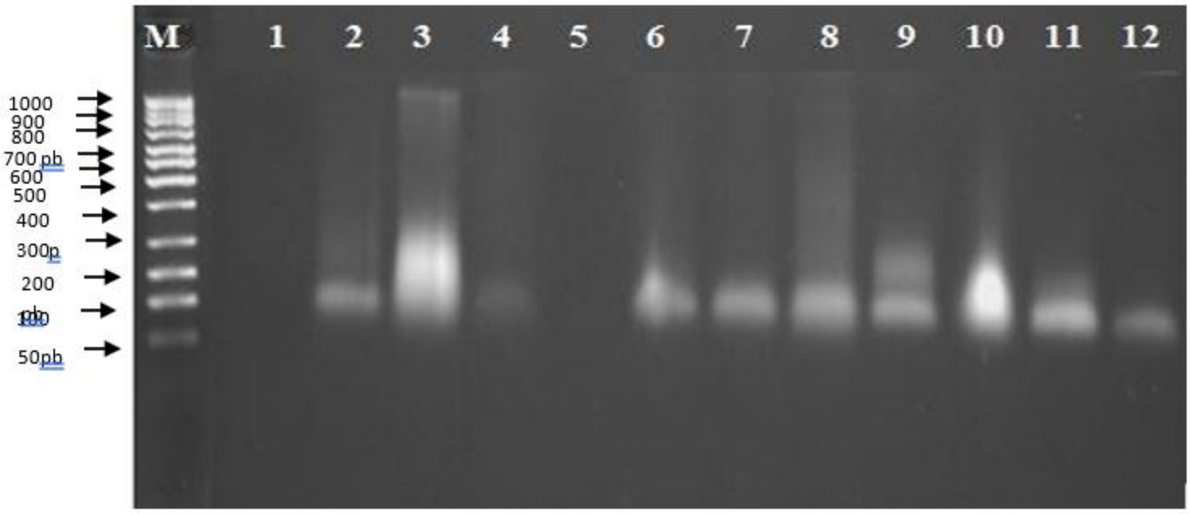


**Figure 1: Shows the Flic d gene reaction pattern, Image showing the results of *Salmonella* analysis**

where the numbers (1–12) represent the sample numbers, M represents the DNA ladder (100 bp)

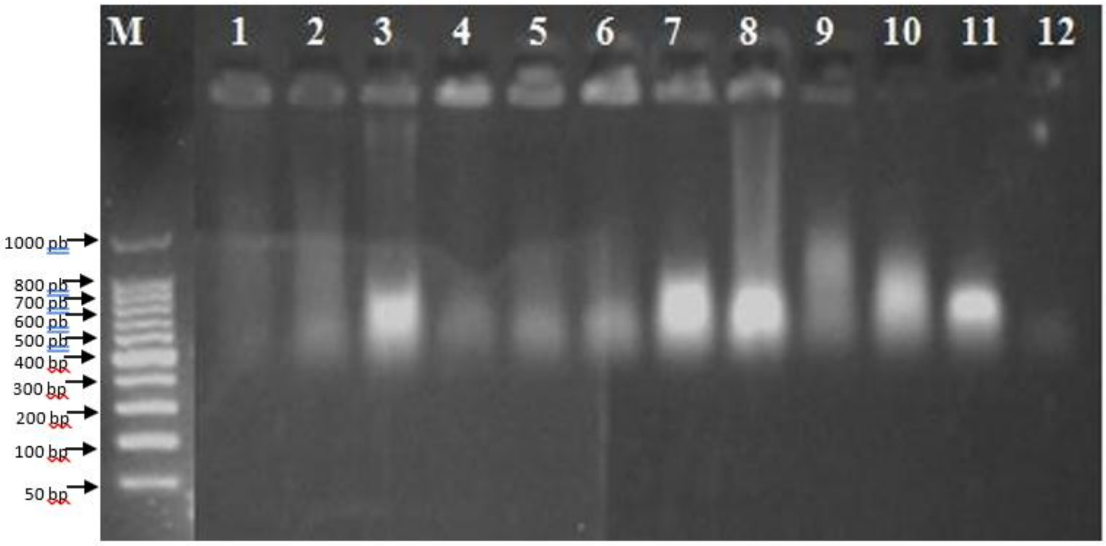


**Figure 2 shows the results for E. coli**

Figure 1 shows the 16S rRNA PCR gel Figure 2 shows the results for E. coli

where the numbers (1–12) represent the sample numbers. M represents the DNA ladder (100 bp)

# DISCUSSION

In this study, 30 chicken meat samples were collected from three different markets and divided into internal and external samples. The results identified six bacterial genera: *E. coli*, *Salmonella spp.*, *Pseudomonas spp.*, *Proteus spp.*, , *Bacillus spp.*, and *Staphylococcus aureus spp.*

## Total Plate Count

The results obtained from the current study showed that the total number of bacterial colonies growing on the Plats Count medium was (2,833,567) CFU/g, of which (932,860) colonies were from the Al-Shabab Market in Khartoum, (1,049,617) from the May Market, and (851,090) from the Omdurman Market

The overall average for these bacteria across the three markets was (944,522).

This study shows that the average bacterial colony count in the current study is ($10^4$× 9.45 cells/gram). The current results were higher compared to a number of previous studies and similar to other studies; specifically, the current results were higher compared to a study conducted by Melhem (2016), where the results did not exceed ($10^3$× 8.53 cells/gram). The high count may be attributed to the failure to follow hygiene protocols in the traditional slaughterhouse , where the person performing traditional slaughter or preparing for slaughter does not wear any protective gear to prevent contamination, such as gloves, and does not perform effective and regular cleaning of the slaughterer's tools and clothing, as reported in the study by Edris2012.. Additionally, most operations performed during traditional slaughter take place in open areas exposed to dust, dirt, and insects.

The current study also shows that bacterial counts are significantly higher in May Market and Market 6 ($10^5$× 1.05 cells/gram) compared to Khartoum's Al-Ashar Market ($10^4$× 8.51 cells/gram) and Omdurman Market, where the highest rate was 10×9.33 cells/gram), respectively. This difference may be due to the lack of hygiene in these traditional slaughterhouses compared to their predecessors, as well as the use of unclean and contaminated tools, in addition to the poor hygiene of the workers inside these slaughterhouses.

The most contaminated of these areas is the Mayu area, due to the potential for contamination resulting from the tools used in slaughtering, plucking, cutting, and cleaning, starting from the slaughtering site—which was on the ground contaminated with poultry feces— through the slaughterer's foot—which is in a shoe—to the table that has not been washed for some time, then the chicken is placed in hot water that has not been changed for some time, and finally to the table that has not been washed for some time to remove the feathers. The removal of heads, feet, and entrails—all of this takes place in the same areas where other poultry are sold and raised.

As for the area with the lowest contamination levels among those under study, it is the Al-Ashra area. This is because the places where the chickens are slaughtered were free of poultry feces, in addition to the cleanliness of the wooden table used to remove feathers and internal organs.

The least contaminated of the areas under study is the Omdurman area, primarily because the sales locations are somewhat distant from the slaughter sites, and the chickens are slaughtered on a ceramic table designed to collect the blood in one place. The slaughterer does not place his foot on the slaughtered chicken, and the feathers are removed and the carcass cleaned on the same table where the slaughter took place, though it is washed every 24 hours. These results are somewhat similar to those of Hoshair (1978), as the bacterial counts on the surface of the chicken ranged between ($10^4$× 100 – $10^4$× 0.05 cells/gram). They also agree with the results reached by Hashim (2005), who observed fluctuations in bacterial counts, particularly Staphylococcus aureus, in chicken meat in markets.Table 6 shows that the study results indicated a high prevalence of certain pathogenic bacteria in the samples studied, reflecting the presence of significant microbial contamination that may pose a health risk to consumers.*E. coli* recorded the highest isolation rate, being found in 27 out of 30 samples. This high rate is a strong indicator of fecal contamination and poor hygiene practices during production and handling; its presence is often associated with poor personal hygiene among workers, contamination of the water used, and inadequate sterilization of equipment. This finding is consistent with numerous studies in developing countries that have reported high rates due to weak health control systems.As for Staphylococcus aureus *(S. aureus)*, it recorded a very high prevalence rate of 93%, having been isolated from 28 samples. This bacterium is one of the most significant causes of food poisoning due to its ability to produce heat-resistant enterotoxins. Its high prevalence often indicates contamination from hands or skin, and this high rate may indicate a potential risk of food poisoning cases.

In the same context, the study showed that *Salmonella spp.* was isolated in 53% of samples (16 samples). It is considered one of the most dangerous causes of foodborne diseases globally, and its presence may be attributed to primary contamination from animals and secondary contamination during distribution. This percentage is high compared to international standards, which require the complete absence of Salmonella in ready-to-eat foods. Additionally, *Proteus spp.* was detected in 26% of 8 samples; these opportunistic bacteria are associated with environmental contamination, and their presence reflects poor environmental hygiene and exposure of the product to inappropriate temperatures. As for *Pseudomonas spp.*, the isolation rate reached 40% (12 samples). These bacteria are known for their ability to spoil food, especially at refrigeration temperatures; their presence indicates weaknesses in the cold chain and post-processing contamination.

As for *Bacillus spp.*, the isolation rate was the lowest at 3%, as it was isolated from only one sample; however, despite this low rate, some *Bacillus* species are capable of forming heat-resistant spores.

The study results confirmed consistency with the study conducted by Ramad in Khartoum State regarding the presence of *Salmonella* in both studies; however, they differ in that this study also detected *E. coli, Proteus, Pseudomonas, Bacillus, Staphylococcus aureus, and Staphylococcus spp*.

In another study conducted by Qutbi in 2011 in Khartoum State, it was found that chicken meat is contaminated with two types of bacteria: *Salmonella* and *E. coli*. These results are similar to those of this study, but they differ in that this study *detected Proteus, Pseudomonas, Bacillus, Staphylococcus aureus, and Staphylococcus sp.*

In another study conducted by Ahmed in 2012 in Khartoum State, chicken meat was found to be contaminated with Salmonella and a group of bacterial species that the researcher did not classify. This study is similar to the present study in terms of the presence of Salmonella but differs in the group of other bacteria.

According to a study conducted by Abdullah in 2013 in Khartoum, the isolated bacteria included Salmonella and some other microbes that the researcher did not classify. However, the isolated Salmonella bacteria were identical in this study, with differences in the other microbes.

In a study conducted by Janssen *et al.* in 2001, the results of bacterial isolation targeting *E.coli*, revealed the presence of various bacterial isolates in addition to *E. coli*, including Gram-negative bacteria such as *Proteus* and *Pseudomonas*. The presence of Gram-positive bacteria such as *Bacillus* and Staphylococcus was also observed. This study was consistent with the results of this research. This is due to differences in the source and number of samples tested, but it may indicate increased health awareness in poultry farming; slaughterhouses also play a significant role in the contamination of carcasses. In a recent study conducted by (Abdul Ali et al., 2013), the authors found that 80% of chicken meat samples tested positive for E. coli, particularly E. coli O123, indicating that contamination cannot be completely prevented during slaughtering processes.

The findings of Hassan (2011) in Iraq showed that the Turkish chicken brand "Dunya Al-Lazah" was the worst, as it contained Salmonella Typhi, Staphylococcus aureus, and *E. coli*.

This study agrees with some of the findings of this research but differs regarding other bacteria such as Pseudomonas, Bacillus, and Proteus. In general, the presence of these microorganisms in the samples under study, given our climatic and environmental conditions and the hot climate, leads to the rapid growth of these bacteria compared to the climatic and environmental conditions of other countries.

It is believed that the presence of these bacteria is due to poor hygiene among workers in chicken slaughterhouses or unsanitary equipment and machinery; sometimes the chickens themselves may be carriers of the disease but are nevertheless slaughtered and consumed.

All bacterial species showed resistance to various antibiotics, and Gram-negative bacteria exhibited high resistance to beta-lactam antibiotics, including ampicillin, ceftazidime, and cefotaxime, in addition to resistance to gentamicin. These results are consistent with those reported by Kaufhold and Klein (1995), who demonstrated that Enterococcus bacteria exhibited high resistance to ampicillin and gentamicin, while the remaining Gram-negative bacteria showed high sensitivity to ciprofloxacin and other antibiotics. These results are consistent with those of Gales, who demonstrated the efficacy of ciprofloxacin against all species of the Enterobacteriaceae family.

Gram-positive bacteria, on the other hand, were resistant to beta-lactam antibiotics—namely ampicillin, ceftazidime, and cefotaxime—and were resistant to tetracycline, co-trimoxazole, and gentamicin, whereas Gram-positive bacteria showed sensitivity to ampicillin and cefuroxime. This result is consistent with Stephan, 1997 et al., who demonstrated that *S. aureus* was highly susceptible to vancomycin. The results of this study, using molecular biology techniques, showed that bacteria isolated from chickens raised in homes and slaughtered traditionally in the state of Khartoum (AlShaabi, May, Omdurman)

A DNA polymerase chain reaction (PCR) test was performed using a primer targeting the 16S rRNA gene located in the chromosomal region, as this gene confirms the identification of these bacteria. After performing the PCR reaction and transferring the results to 1.5% agarose gel, followed by UV examination, bands with a molecular weight of 400 base pairs appeared for 12 bacterial isolates, indicating the presence of the target gene in these isolates. Figure 1. Meanwhile, the isolates labeled with numbers (1 and 12) did not carry any copy of this gene, confirming the results of diagnosis using traditional biochemical methods that these isolates belong to *E.coli*. The results also showed that this gene is a dominant trait in all E. coli strains, meaning that these bacteria possess the gene carrying the genetic code specific to the diagnostic trait of 16S rRNA. Thus, these results indicate that this is a diagnostic gene for these bacteria, as its results match those of traditional diagnostic tests, thereby rendering them obsolete and allowing this gene to be used for diagnosing these bacteria.

This saves time and effort and provides high accuracy in diagnosing this bacterium. Molecular diagnosis of bacterial isolates is important and yields highly sensitive and accurate results in the field of diagnosis. Since diagnosis via biochemical tests can yield false-positive results, the current study focused on using molecular tests through the amplification of the 16S rRNA gene. The results of this study were consistent with those reported by Youssef et al., 2015, who found that all studied samples contained the 16S rRNA gene. Based on the above results, molecular diagnosis using 16S rRNA is considered appropriate and provides highly accurate and sensitive results in bacterial diagnosis, in addition to saving the time and effort required by the conventional diagnostic process. Meanwhile, the results confirmed the diagnosis of Salmonella using the FLIC-D gene primer to identify bacteria belonging to the genus *Salmonella* isolated from chicken meat, yielding typical colonies with a gene length of 300 bp upon amplification of the (Flic-d gene), it was confirmed from this gene that these bacteria belong to the genus Salmonella. After performing DNA amplification and transferring the results of this process onto agarose gel and examining it under UV light, bands with a molecular weight (300 bp) for the bacterial isolates under study, indicating the presence of the target gene in these isolates, as shown in Figure 2. Figure 2 shows bands of 300 bp in length relative to the DNA molecular weight standard (100 bp Ladder) in lane M.Meanwhile, the isolates labeled 1 and 5 did not carry any copy of this gene, confirming the results of conventional diagnostic methods that these isolates do not belong to Salmonella Typhi. These results were found to match those in the National Center for Biotechnology Information (NCBI) database.

## CONCLUSIONS

- The bacterial species *Salmonella spp., E. coli, and* S. *aureus spp.* are the most common in home-raised chicken meat
- The wide variety of bacterial species indicates that contamination has multiple sources (human, environmental, and water-borne).
- The antibiotics to which the bacteria showed the highest resistance were Ampicillin and Co-Trimoxazole, while other antibiotics were effective against all types (Gram-negative and Gram-positive) under study.
- The Polymerase Chain Reaction (PCR) technique has proven its effectiveness as a rapid and highly accurate tool for the specific detection of Salmonella, due to its high sensitivity and specificity compared to traditional methods relying on morphological and biochemical characteristics.
- Carcasses are exposed to bacterial contamination during and after slaughter from various sources, including tools, hands, and during the plucking of feathers from the carcass. See the image

## RECOMMENDATIONS

1. It is necessary to improve traditional slaughter conditions by implementing hygiene and sterilization standards in slaughterhouses, particularly in Khartoum.
2. Adherence to good hygiene practices during evisceration to prevent the spread of contamination from the intestines to the rest of the carcass.
3. Strengthen routine veterinary inspections of slaughterhouses and local markets.
4. Health awareness campaigns for vendors and consumers regarding the risks of pathogenic bacteria transmitted through poultry.
5. Adopting PCR technology as a rapid and accurate diagnostic tool for detecting Salmonella instead of relying solely on traditional methods.
6. Limit the indiscriminate use of antibiotics in poultry farming.
7. Conducting expanded studies that include a larger number of samples and different geographic regions within Chad and Sudan.
8. Study the genetic factors responsible for the virulence of the isolated bacteria using molecular biology techniques.

Image No. 1

Image No. 2

Image No. 3

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