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# Molecular Characterisation and Antimicrobial Resistance of Pathogenic *Escherichia coli* Isolated from Chicken Meat in Chennai, Tamil Nadu, India

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**Abstract**
Inappropriate use of antimicrobials has led to the development, recurrent emanation and dissemination of resistant bacteria, and as a consequence, antibiotic resistance has become a scourge of the poultry industry. A total of 200 chicken meat samples were collected from retail meat outlets from local markets in and around Chennai, Tamil Nadu, India and the antibiotic susceptibility of *Escherichia coli* isolated from them was assessed. Gene-specific primers (uspA for *E. coli)* was used to characterise the isolated bacteria by molecular methods to confirm the presence of pathogenic *E. coli* in the meat samples. The isolates' susceptibility to commercially available antibiotics was evaluated by the Kirby-Bauer disc diffusion method. The isolates were grouped as susceptible, intermediate, or resistant, based on the measurement of zones of inhibition, following CLSI standards and. The results revealed a prevalence of *E. coli* in 65% of broiler chicken meat samples, highlighting the importance of *E. coli* as a potential pathogen in meat borne illness. The *E. coli* isolated in the study exhibited muli-drug resistance emphasising the need to exercise discretion in use of antibiotics use and institute suitable measures to bolster food safety so as to ensure the constant decline in the prevalence of antibiotic resistance in the poultry industry.

**Keywords:** *E. coli*, chicken meat, molecular characterisation antimicrobial, antimicrobial susceptibility, antimicrobial resistance, disc diffusion method

## Introduction
Foodborne illnesses are a significant public health concern worldwide. Poultry products, including chicken meat have been identified as significant potential sources of various pathogenic bacteria, and is commonly incriminated in outbreaks of foodborne illness. *Escherichia coli* (*E. coli*) has emerged as a critical food-borne pathogen due to its potential to act as an infectious agent, given its very low infective dose, and its mounting resistance to antimicrobial agents. The evolution of antimicrobial resistance in food-borne bacteria has become a major challenge in food safety and has warranted comprehensive studies to understand the molecular characteristics and antimicrobial susceptibility profiles of these pathogens. Meat serves as a highly probable route for the transmission of antibiotic-resistant strains, including those resistant to multiple antibiotic groups, to humans (Smet *et al*., 2010) [10]. The antibiotic susceptibility test, also known as the disk diffusion method, assesses the bacterial response to specific antibiotics. Paper discs infused with antibiotics are placed on agar plates inoculated with bacteria. The zone of inhibition around the discs indicates susceptibility, measured to determine antibiotic effectiveness (CLSI, 2018). Further, *uspA* gene is used for the identification of *E. coli* and confirmation of pathogenicity in various samples. The present study aims to investigate the existence and pattern of antimicrobial resistance of pathogenic *E. coli* isolated from chicken meat samples during 2022-2023. To detect *E. coli*, the Polymerase Chain Reaction (PCR) technique, which is a powerful molecular tool capable of detection and molecular characterization of specific genes associated with bacterial isolates (Liu *et al*., 2016) [5] was employed, apart from conventional culture methods.

## Materials and Methods

### Sample collection

Chicken meat swab samples (N=200) were aseptically procured from various retail meat outlets situated in Chennai (including its suburbs), Tamil Nadu, India during 2022-2023. The samples were packed in sterile polythene bags and hygienically transmitted to the Department of Livestock Products Technology (Meat Science), Madras Veterinary College, Chennai-7 in a insulated ice box, which was pre-cleaned thoroughly.

### Bacterial Isolation and DNA extraction

About 25 g of broiler chicken meat sample was taken in a stomacher bag and Two hundred and twenty-five ml (225ml) of sterile buffered peptone water (BPW) was poured into the bag and was then subjected to homogenization at 230 revolutions per minute (rpm) for 30 seconds using a stomacher. The homogenate of 1ml was transferred into Tryptone soya broth (TSB) and placed in an incubator set at 37 ºC for 18-24 hours. A loopful of the bacterial culture was then streaked on a selective media, Eosin methylene blue and the petri dish placed in an incubator, set at 37 °C for 24 hours, to promote the selective isolation of *Escherichia coli*. Subsequently, morphologically similar, and predominant bacterial colonies were chosen and inoculated in nutrient broth for 24 hours at 37 °C to achieve and maintain pure cultures.

### Molecular Characterization

The DNA was isolated from pure culture using the Qiagen kit method as per the manufacturers instructions. The gene-specific oligonucleotide primer targeting (usp A) gene was used for confirmation of bacterial species (pathogenic *E. coli)* in this study are given in Table 1. In each 25 µL PCR reaction, 2 µL of template DNA, 1 µL (10µM) of forward and reverse primers each, 12.5 µL red dye master mix (2x) and 8.5 µL nuclease-free water were added. The PCR product was run on 1% agarose gel electrophoresis and examined visually under a UV transilluminator.

### Antibiotic Susceptibility Testing

Kirby-Bauer disc diffusion method was used to assess the antibiotic susceptibility of the isolated strains. Organisms that were subjected to assessment were suspended in normal saline to 0.5 McFarland standard and then incubated overnight at 37 ºC for 18 to 24 hr. The antimicrobials used in the study were ampicillin, ceftriaxone, ciprofloxacin, gentamicin, sulphafurazole, and tetracycline. One loop full of bacterial isolates was streaked on the entire surface of selective agar plates. Then the antibiotic discs were loaded on the appropriate plates. The plates were then placed in an incubator set at 37 °C for 24-48 hours. The diameters of the zone of inhibition were measured in millimeters (mm) and recorded. According to their response to the antimicrobial drugs, the organisms were identified as susceptible (S), intermediate (I), and resistant (R) as per CLSI, 2018.

## Results and Discussion

The 200 chicken meat swab samples collected from different regions in Chennai were subjected to the studies. Two different bacterial isolates were identified after inoculation with the selective medium. The isolates were further confirmed using PCR amplification targeting species-specific genes for the presence of *E. coli* with amplicon sizes of 884 bp (Figure 1). The prevalence percentage of bacterial isolates in the chicken meat samples evaluated was 130/200 (65%). Mandal *et al.* [1] have recorded a prevalence of 86% in broiler chicken in Bangladesh.

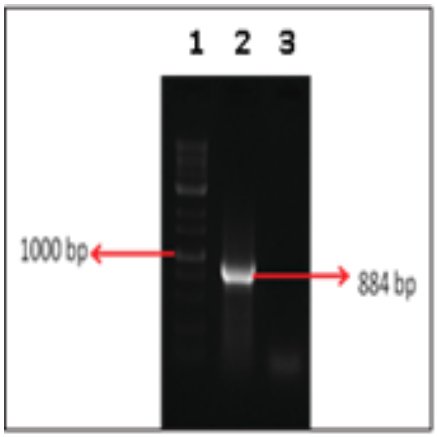


**Fig 1:** Agarose gel electrophoresis (1% agarose) of PCR amplified products using gene-specific (usp A) PCR primer for *Escherichia coli* from chicken meat sample. Lanes 1-1 kb DNA ladder, 2-Test positive DNA sample (amplicon size: 884 bp), 3-No template control

**Table 1:** Details of primers sequence used for amplification of Usp A gene of *E. coli*

| Genes | Primers | Product size | Reference |
|---|---|---|---|
| Usp A (*E. coli*) | F:CCGATACGCTGCCAATCAGT R:ACGCAGACCGTAGGCCAGAT | 884 | Chen and Griffiths, (1998) |

In this present study, *E. coli was resistant to* ampicillin (73.07%), ciprofloxacin (93.07%), tetracycline (90.7%), and ceftriaxone (35%). The present study was incongruent with Smet *et al.* (2010) [10] who conducted studies on Ceftiofur-resistant β-lactamase-producing *E. coli* isolates from 5-week-old broilers on five different farms of Belgium and reported that 73.9% of the isolates were resistant to ceftriaxone and 48.1% of the isolates were resistant to tetracycline respectively. Brower *et al.* (2017) [1] have reported the presence of multi-drug resistance in *E. coli* isolates from chicken samples collected from broiler farms (94%) and layer chicken farms (60%). Saud *et al.* (2019) [8] observed that bacteria isolated from chicken meat displayed higher antimicrobial resistance rates in comparison to those isolated from buffalo meat, specifically exhibiting elevated resistance against antimicrobials such as Amoxicillin, Tetracycline, Cotrimoxazole and Nalidixic acid. A review on the bacteriological quality of poultry meat in Nepal reported resistance in, such as *E. coli*, *Staphylococcus*, and *Klebsiella*, towards commonly used antibiotics found in the market (Neupane, 2019) [7].

The Centre for Science and Environment (2017) [2] conducted a study in India on broiler poultry farms, revealing a concerning trend of antibiotic resistance. Multidrug resistance was found to be high in the poultry environment (poultry litter, poultry farm soil and nearby agricultural soil). The highest resistance overall was observed in *E. coli*, followed by *K. pneumoniae* and *S. lentus*.

Antibiotic susceptibility assay in the present study also shows multi-drug antibiotic resistant *E. coli* in chicken meat samples, which should be reckoned as a clarion call for responsible antibiotic use in poultry farming and stringent food safety. Continued monitoring is essential to combat resistance and protect public health.

**Table 2:** Bacterial isolates (*E coli*) and their resistant percentages for various antibiotics

| Drug | %Resistant |
|---|---|
| Ampicillin | 73.07 |
| Ceftriaxone | 35.38 |
| Ciprofloxacin | 93.07 |
| Gentamicin | 46.92 |
| Sulphafurazole | 36.15 |
| Tetracycline | 90.76 |

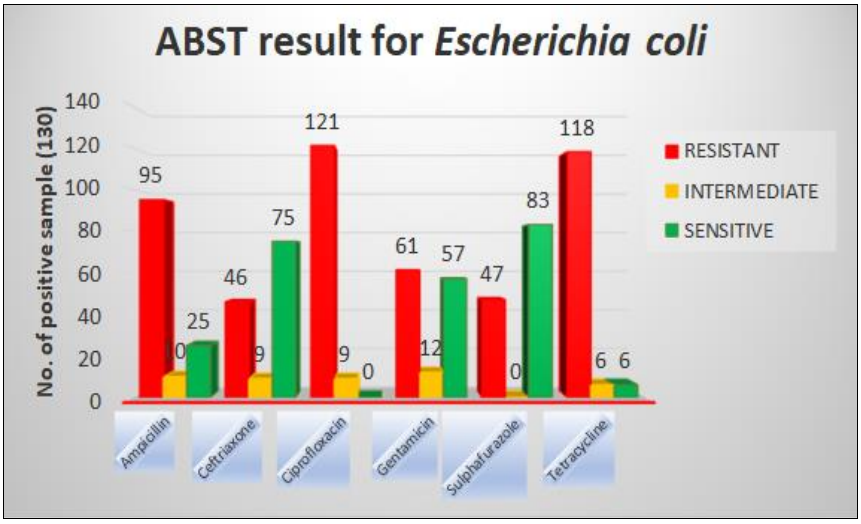


**Fig 2:** ABST results for *E. coli*

## Conclusion

The result highlights the presence of multi-drug resistant pathogenic bacterial strains of *Escherichia coli* in meat samples acquired from retail meat shops in Chennai district, which will induce the spread of resistant strains in the food chain causing adverse health effects. The resistant strains exhibit resistance to widely used antibiotics in multiple countries. These strains have higher tendency to impart similar resistance on humans upon consumption.

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## Conflict of Interest

Not available

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Not available

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