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# Antimicrobial susceptibility testing of *Escherichia coli* isolated from chicken meat in Hisar, Haryana

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**Abstract**
Multi-drug resistant *E. coli* is a serious issue that is becoming increasingly concerning for global public health. The aim of this study was to investigate the antibiotic resistance pattern of *E. coli* isolates obtained from raw chicken meat samples collected from local market of Hisar district of Haryana. Kirby-Bauer disk diffusion susceptibility testing was used to determine the antimicrobial resistance pattern of the *E. coli* isolates. The *E. coli* isolates were highly resistant to penicillin (98.41%), erythromycin (98.41%), amoxicillin-clavulanic acid (93.65%), cefpodoxime (93.65%), tetracycline (93.65%), imipenem (90.4%), ceftazidime (87.30 %) and cefotaxime (85.71 %). Nearly, all the *E. coli* isolates (98.4%) belonged to multi drug resistant bacteria. A total of thirty-four Resistor types were observed during the present study. To reduce the foodborne pathogen contamination, novel, practical, efficient food safety controls and surveillance methods of multi-drug resistance foodborne pathogens are needed.

**Keywords:** *Escherichia coli*, antimicrobial resistance, food samples, chicken meat

## 1. Introduction

As a result of exponential rise population, global biomass of humans overtakes the global biomass of animals raised for food. In order to fulfill this demand, developing countries are shifting towards highly cost-efficient and vertically integrated intensive livestock production systems [1]. Because antimicrobials are necessitate to keep animals healthy and to enhance productivity in these production system, as a result in increase in antimicrobial consumption as well as AMR bacteria. This increase in use of antibiotics has been attributed to a variety of contributing reasons, including the use of clinical antibiotics in farm animal feeds, the use of antibiotics to promote farm animal growth, and the excessive use of antimicrobials in both humans and animals [2]. Antimicrobial resistance (AMR) is one of the most serious threats for both public and animal health worldwide, inadequate selection and abuse of antimicrobial agents in humans and animals being one of the main causes of this problem [3]. One of the major public health issues has been the rise of multidrug resistant (MDR) foodborne bacteria. Multiple drug resistance (MDR) is understood to represent the development of resistance to at least one antimicrobial agent across three or more types of antibiotics [4]. MDR *E. coli* has been recognized as one of the most significant challenges in food safety [5]. Emergence of resistance to most first line antimicrobials agents, treatment for *E. coli* infection has been increasingly. Over the years, resistance to beta lactam antimicrobial among members of *Enterobacteriaceae* has increased mainly due to the spreading of extended spectrum beta lactamases (ESBLs) [6]. The purpose of this study was to determine the antimicrobial susceptibility patterns in *E. coli* strains isolated from chicken meat samples collected from local market of Haryana.

## 2. Material and Methods

### 2.1 Collection of *E. coli* isolates

A total of 63 morphological distict *E. coli* isolates were obtained from 50 chicken meat samples collected from local market of Hisar district of Haryana. The confirmation of *E. coli* isolates both biochemically and molecularly was done previously [7].

### 2.2 Antimicrobial susceptibility testing

The bacterial isolates were subjected to in-vitro antibiotic sensitivity test as per the standard agar disk diffusion method according to CLSI (clinical and laboratory standards institute) [8] using commercially available fifteen antimicrobial disks (HiMedia laboratories Limited, Mumbai) of eight different antibiotics classes.

Isolates were screened for susceptibility to amoxicillin-clavulanic acid (AMC) (30 µg), penicillin (P) (10 µg), cefoperazone (CPZ) (75 µg), cefpodoxime (CPD) (10 µg), cefotaxime (CTX) (30 µg), ceftazidime (CAZ) (30 µg), ceftriaxone (CTR) (30 µg), erythromycin (E) (15 µg), amikacin (AK) (30 µg), gentamicin (GEN) (10 µg), streptomycin (S) (10 µg), chloramphenicol (C) (30 µg), tetracycline (TE) (30 µg), aztreonam (AT) (30 µg) and imipenem (IPM) (10 µg) by the disk diffusion assay in Mueller-Hinton agar. Single isolated bacterial colony from pure fresh culture was transferred into brain heart infusion broth (BHI) and incubated at 37 °C for 6 h. The test broth was adjusted to McFarland 0.5 turbidity to obtain desired bacterial population. Plates of Muller Hinton Agar (MHA), pH 7.2-7.4 were inoculated with inoculum with the help of a sterile cotton swab. The surface of media was uniformly inoculated with the help of swab to ensure even distribution. After the plates dried, antibiotic disks were placed on the inoculated plates using sterile forceps. The antibiotic disks were gently pressed onto the agar to ensure firm contact with the agar surface, and incubated at 37 °C for 24 h. Next day the diameter of inhibition zone formed around each disk was measured using transparent ruler by lying it over the plates. The results were classified as sensitive, intermediate or resistant according to the standardized table supplied by CLSI, 2012.

### 2.3 Statistical analysis

All the statistical analysis were carried out using STATA™ IC -15.0 (StataCorp, College Station, TX).

## 3. Results

### 3.1 Antimicrobial susceptibility testing of *E. coli* isolates

All sixty-three *E. coli* isolates were tested for their antimicrobial resistance against eight different common classes of antibiotics and fifteen different commercial antibiotics. The results of phenotypic resistance tests to antibiotics of the isolates are shown in Table 1 and Fig. 1. Penicillin, erythromycin, amoxicillin-clavulanic acid, cefpodoxime, tetracycline and imipenem were found to be resistant in more than 90% of *E. coli* isolates. However, the lowest antibiotic resistance phenotypes were observed against chloramphenicol (29; 46.03%), amikacin (35 isolate; 55.56%), gentamicin (35 isolate; 55.56%) and aztreonam (42 isolate; 66.67%).

A total of 62 (98.41%: 95% CI- 91.47-99.96%) *E. coli* isolates showed resistance to at least three different classes of antimicrobial agents and were considered as MDR *E. coli* isolates. Thirty-four resistor types were observed during the present study, details of which are given in Table 2.

**Table 1:** Antimicrobial susceptibility profile of *E. coli* isolates obtained from chicken meat (n=63)

| Antibiotic | Resistant | Intermediate | Sensitive |
|---|---|---|---|
| AMC | 59 (93.65) | 0 (0.00) | 4 (6.35) |
| P | 62 (98.41) | 0 (0.00) | 1 (1.59) |
| CTX | 54 (85.71) | 7 (11.11) | 2 (3.17) |
| CPD | 59 (93.65) | 1 (1.59) | 3 (4.76) |
| CPZ | 48 (76.19) | 8 (12.70) | 7 (11.11) |
| CAZ | 55 (87.30) | 0 (0.00) | 8 (12.70) |
| CTR | 47 (74.60) | 4 (6.35) | 12 (19.04) |
| AT | 42 (66.67) | 7 (11.11) | 14 (22.22) |
| IPM | 57 (90.48) | 3 (4.76) | 3 (4.76) |
| TE | 59 (93.65) | 2 (3.17) | 2 (3.17) |
| E | 62 (98.41) | 0 (0.00) | 1 (1.59) |
| AK | 35 (55.56) | 15 (23.81) | 13 (20.63) |
| GEN | 35 (55.56) | 5 (7.94) | 23 (36.51) |
| S | 44 (69.84) | 12 (19.04) | 7 (11.11) |
| C | 29 (46.03) | 15 (23.81) | 19 (30.16) |

**Table 2:** Resistor types of *E. coli* isolates (n=63)

| No. of antimicrobial to which resistant | Resistor types | No. of isolates |
|---|---|---|
| 15 | AM-P-CT-CP-CZ-CA-CR-AT-IPM-T-E-AK-G-S-C | 12 |
| 14 | AM-P-CT-CP-CZ-CA-CR-AT-IPM-T-E-AK-G-C | 2 |
| | AM-P-CT-CP-CZ-CA-CR-AT-IPM-T-E-AK-G-S | 6 |
| | AM-P-CT-CP-CZ-CA-CR-AT-T-E-AK-G-S-C | 2 |
| | AM-P-CT-CP-CZ-CA-CR-AT-IPM-T-E-AK-S-C | 1 |
| | AM-P-CT-CP-CZ-CA-CR-IPM-T-E-AK-G-S-C | 2 |
| 13 | AM-P-CT-CP-CZ-CA-CR-AT-IPM-T-E-AK-G | 2 |
| | AM-P-CT-CP-CZ-CA-CR-AT-IPM-T-E-AK-S | 2 |
| | AM-P-CT-CP-CZ-CA-CR-AT-IPM-T-E-G-C | 3 |
| | AM-P-CT-CP-CZ-CA-CR-AT-IPM-T-E-S-C | 1 |
| | AM-P-CT-CP-CZ-CA-CR-AT-T-E-AK-G-S | 1 |
| | AM-P-CT-CP-CZ-CA-CR-IPM-T-E-AK-G-S | 2 |
| 12 | AM-P-CT-CP-CZ-CA-CR-AT-IPM-T-E-AK | 1 |
| | AM-P-CT-CP-CZ-CA-CR-AT-IPM-T-E-C | 2 |
| | AM-P-CT-CP-CZ-CA-CR-AT-IPM-T-E-G | 1 |
| | AM-P-CT-CP-CZ-CA-CR-AT-IPM-T-E-S | 3 |
| | AM-P-CT-CP-CZ-CA-CR-IPM-T-E-AK-S | 1 |
| | AM-P-CT-CP-CZ-CA-CR-IPM-T-E-S-C | 1 |

| | | |
|---|---|---|
| 10 | AM-P-CP-CA-IPM-T-E-G-S-C | 1 |
| | AM-P-CP-CZ-CA-CR-IPM-T-E-S | 1 |
| | AM-P-CT-CP-CA-CR-AT-IPM-T-E | 1 |
| | AM-P-CT-CP-CZ-CA-IPM-T-E-S | 1 |
| 9 | AM-P-CP-CZ-IPM-T-E-G-S | 1 |
| | AM-P-CT-CP-CA-IPM-T-E-S | 3 |
| 8 | AM-P-CT-CA-IPM-T-E-S | 1 |
| | AM-P-CT-CP-IPM-T-E-S | 1 |
| 7 | AM-P-CP-IPM-E-AK-G | 1 |
| | AM-P-CP-IPM-T-E-C | 1 |
| | AM-P-CP-IPM-T-E-S | 1 |
| 6 | AM-P-CP-IPM-T-E | 1 |
| 4 | P-AT-E-C | 1 |
| | P-CP-T-E | 1 |
| | P-CT-CA-E | 1 |
| 3 | CT-CA-AT | 1 |

* AM-Amoxicillin-clavulanic acid, P-penicillin, CT-Cefotaxime, CP-Cefpodoxime, CZ Cefoperazone, CA-Ceftazidime, CR-Ceftriaxone, AT-Aztreonam, IPM-Imipenem, T-Tetracycline, E-Erythromycin, AK-Amikacin, G-Gentamicin, S- Streptomycin, C- Chloramphenicol

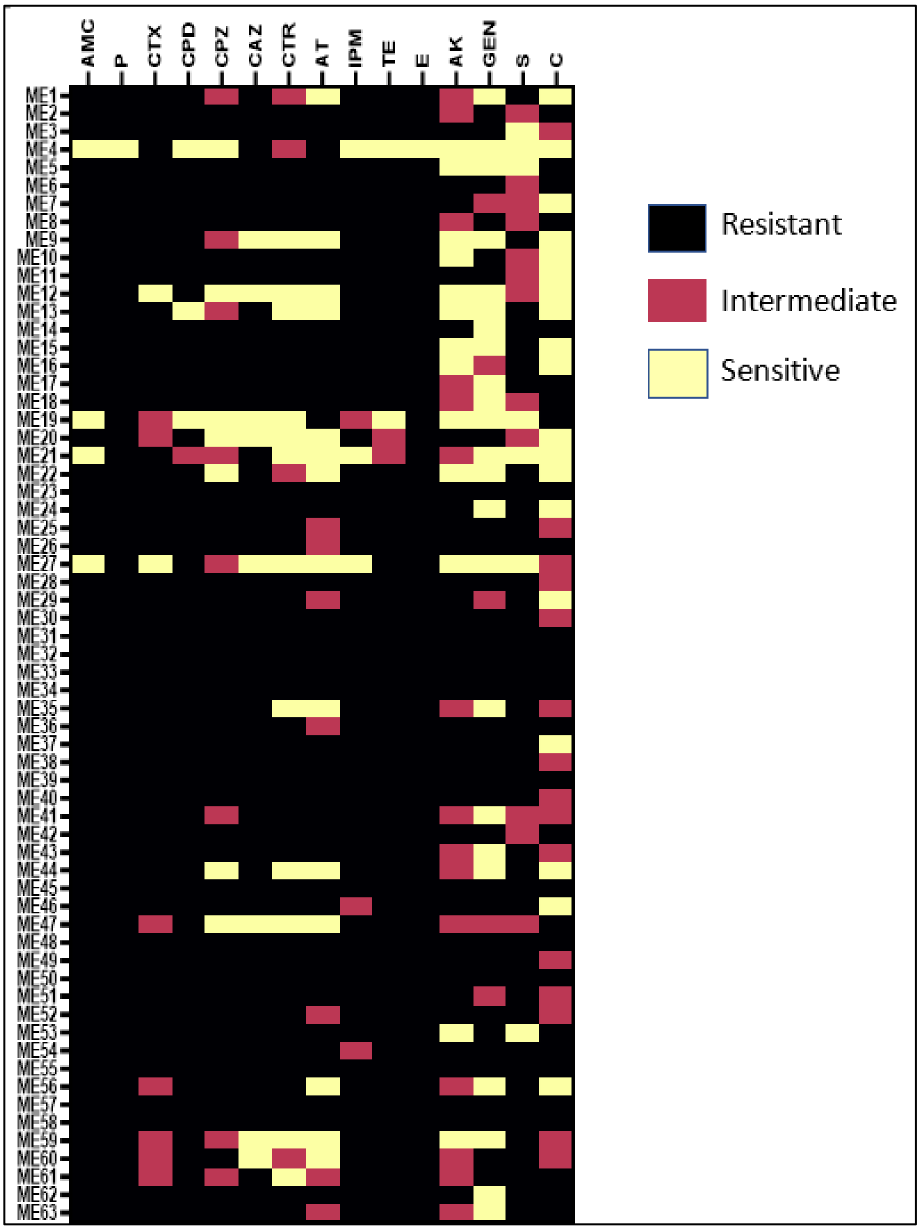


**Fig 1:** Heat map of antibiotic susceptibility profile of *E. coli* isolates (n=63)

## 4. Discussion

A wide range of antimicrobial medications are currently being used worldwide for growth promotion, diseases prevention and treatment of sick poultry flock allowing the development of MDR foodborne pathogens [9]. As per the available literature, enrofloxacin, gentamicin, amikacin, amoxicillin, tetracyclines, and ampicillin were the most commonly used antibiotics in chicken farms, while ceftiofur, ceftriaxone, and nitrofurantoin were among the less frequently used antibiotics [10]. The majority of current treatment strategies focus on early prevention of the disease in the broiler flocks rather than treatment after infection [11]. It results in raising serious concerns for human health and the poultry industry's bottom line [12]. One of the main concerns in food safety and public health is the emergence of antibiotic resistant foodborne bacterial pathogens [13]. Several studies have reported that foods of animal origin might be an important source of human-acquired MDR pathogenic *E. coli* [5] (Rashid *et al*., 2013). Poultry and meat products can be widely contaminated with pathogenic or non-pathogenic groups of *E. coli* of animal origins, including MDR strains.

In the present study *E. coli* isolates showed more than 90% resistance for penicillin, erythromycin, amoxicillin-clavulanic acid, cefpodoxime tetracycline and imipenem. Among all *E. coli* isolates, 93.65% showed resistant against tetracycline and the similar findings have been observed in other studies [6, 14, 15, 16, 17] in India and other parts of the world. It was observed that 90.48% isolates of meat origin in present study were resistant to imipenem, while in some studies [6, 18] contradictory results have been reported in which all the isolates from meat samples were sensitive to imipenem. High resistance observed in this study especially in isolates of meat origin is of great concern and indicates use of this class of drug in poultry production which is otherwise is not to be used in animals.

WHO prepared a list of critically important antimicrobial for human medicine, which implies that they should be the sole, or one of limited available therapies, to treat serious bacterial infections in people. Some of the “Critically important" antimicrobials have been further designated as “Highest priority critically important" [19]. Cefoperazone, cefotaxime, ceftazidime, cefpodoxime and ceftriaxone (third generation cephalosporins) and erythromycin (macrolides) from our study are included in the category of “Highest priority critically important" antimicrobials and besides these others antimicrobials like penicillins, aminoglycosides, imipenem and aztreonam are “Critically important" antimicrobials. High resistance to these antimicrobials as observed in our study is of concern both for clinical use of them in human infection and transmission of resistance to humans which is a serious concern from public health point of view.

## 5. Conclusion

This study showed that *E. coli* isolates were highly resistant to penicillin, erythromycin, amoxicillin-clavulanic acid, cefpodoxime, tetracycline, imipenem, ceftazidime and cefotaxime antibiotics. We found that 98.4% of *E. coli* isolates were MDR to at least three classes of antimicrobial agents. To control and prevent foodborne pathogen contamination and diseases, there is an urgent need of new and effective food safety control and surveillance systems and genotyping of foodborne pathogens, especially MDR strains.

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