Prevalence and characterisation of antimicrobial resistance pattern of ESBL-producing *Escherichia coli* isolated from poultry in Banaskantha district, India

MITUL A PATEL1*, APARNA PANDEY1, SANDIP S PATEL2, ARUN C PATEL2, SUSHIL K MOHAPATRA2, HARSHAD C CHAUHAN2, KISHAN K SHARMA2, BHAVESH I PRAJAPATI1 and BHARATSINGH S CHANDEL2

Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Banaskantha, Gujarat 385 505 India

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**ABSTRACT**

Resistance to antimicrobial drugs among bacterial pathogens is an emerging problem. Bacterial resistance to beta-lactam antibiotics has grown significantly. The present study aims at isolation, identification, phenotypic confirmation of ESBL-producer, and antimicrobial resistance pattern of *Escherichia coli* isolated from poultry faecal samples. A total of 120 samples were collected from 30 different poultry farms in Banaskantha district. 108 out of 120 samples were confirmed as *E. coli* using microscopic, biochemical tests, followed by species-specific 16S rRNA PCR; among them, broiler 65 (92.85%) and layer 43 (86%) were confirmed as *E. coli*. The phenotypic ESBL-production was detected in 54 (50%) isolates with broiler (29.62%) and layer (20.28%) farms. The antibiotic sensitivity pattern revealed the highest resistance was identified against Cefpodoxime 100%, followed by Tetracycline 97.22%, Amoxicillin + Clavulanic acid 95.37%, Nalidixic Acid 94.44%, Enrofloxacin 91.66%, Co-trimazazole 90.74%, Ampicillin 78.70% and Amikacin 57.40%. The 95.37% and 77.77% isolates showed susceptibility to Imipenem and Cefoxitin, respectively. Thus, it may be concluded that in the current study, isolates revealed multidrug resistance against antimicrobial agents commonly used in veterinary and human practice. This implies that the existing practice of misuse and improper use of antibiotics in poultry accelerates antimicrobial resistance in poultry.

**Keywords:** Antibiotics, Antimicrobial resistance, *E. coli*, India, PCR, Poultry

Antimicrobial resistance is caused by developing and disseminating multidrug-resistant bacteria that have developed new resistance mechanisms and are becoming a growing threat to public health (Racewicz et al. 2022). Misuse and overusage of antibiotics in livestock have been associated with the emergence and spread of bacterial resistance (Manyi-Loh et al. 2018). In India, the poultry industry is fastest growing in the agriculture sector. India is the world’s fourth-largest producer of chicken and eggs after China, Brazil, and the US (APEDA). Globally, many poultry farmers often use low doses of antibiotics as growth-promoting agents and preventive chemotherapy (Van et al. 2019). It has been estimated that 80% of food-producing animals and birds received antibiotics throughout the majority of their lives (Husain et al. 2021). India is among the top consumers worldwide, accounting for 3% of global consumption of antibiotics.

*Escherichia coli* is a commensal bacterium found in the intestine’s natural flora. Some strains of *E. coli* are a frequent cause of intestinal and urinary tract infections in animals and humans (Ramos et al. 2020) and are transmitted via the fecal-oral route. *E. coli* causes disease in poultry, generally referred to as avian pathogenic *E. coli* (APEC). Nowadays, the emergence of extended-spectrum beta-lactamase (ESBL) is increasing globally. ESBL is an enzyme commonly found in gram-negative bacteria and inactivates the many antibiotics used to treat bacterial infections. ESBL *E. coli* has resistance to third-generation cephalosporin and quinolones antibiotics (Kettani et al. 2021).

Antimicrobial resistance is one of the top ten global health issues for 2019, and according to the World Health Organization (WHO) India has among the highest bacterial infections in the world. Resistance is rapidly increased by spreading resistance genes using mobile genetic elements or horizontal gene transfer mechanisms (Patel et al. 2022). In the last two decades, AMR in animals has grown by 50%, compared to several years’ usual approval time for new antibiotics (Boeckel et al. 2019). In recent years, several studies have identified the prevalence of beta-lactamase antibiotic resistance in *E. coli* from poultry (Tansawai et al. 2019). Therefore, the study aimed to isolate phenotypic confirmation of ESBL-production and antimicrobial
resistance profile of *E. coli* isolated from poultry farms (broiler and layers) in Banaskantha, India.

**MATERIALS AND METHODS**

*Study area and sample collection:* Cloacal swabs (120) were randomly collected from 30 different poultry farms (four from each farm) in semi-arid climate area of Gujarat. Following aseptic precautions, sterile swab was gently inserted into the cloaca of the chicken and carefully swabbed on the mucosal wall several times. Samples were packed carefully and transported immediately to a laboratory at 4°C. The sick and dead chickens (broiler and layer) were eliminated from the study.

*Isolation and identification of *Escherichia coli:** Primary isolation was done by inoculation of the cloacal swab into MacConkey broth for pre-enrichment and incubated at 37°C for 24 h. Then the incubated culture was streaked on MacConkey agar and incubated at 37°C for 24 h. Lactose fermenting pink presumptive colonies were purified by streaked on Eosin Methylene Blue (EMB) agar for selective isolation. The plates were incubated at 37°C for 24 h, and colonies showing a greenish metallic sheen were considered confirmatory for *E. coli*.

The microscopic morphology of the isolates was studied by Gram’s staining method, followed by biochemical tests like Oxidase, catalase, Indole, Methyl red, Voges-Proskauer, and Citrate were employed to confirm *E. coli* isolates were identified using species-specific 16S rRNA gene amplification as per the method described by Fratamico *et al.* (2000). The PCR reaction was prepared by adding 12.5 µl master mix, 2 µl primer, 1 µl of each ECO-1 forward primer :- 5’GACCTCGGTTTAGTTCACAGA 3’, and then 8.5 µl nuclease free water was added to get a total volume of 25 µl. The PCR cyclic condition for ECO-1 was initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 53°C for 1 min, an extension at 72°C for 1 min, and a final extension at 72°C for 8 min.

*Genotypic confirmation of *E. coli* isolates using PCR method:* The DNA was extracted from the presumptively confirmed bacterial colonies using the DNeasy Blood and Tissue Kit (Qiagen), following the manufacturer’s protocols. *E. coli* isolates were identified using species-specific 16S rRNA gene amplification as per the method described by Fratamico *et al.* (2000). The PCR reaction was prepared by adding 12.5 µl master mix, 2 µl DNA template, 1 µl of each ECO-1 forward primer :- 5’CACACGCTGACGCTGACCA 3’, and reverse primer :- 5’GACCTCGGTTTAGTTCACAGA 3’, and then 8.5 µl nuclease free water was added to get a total volume of 25 µl. The PCR cyclic condition for ECO-1 was initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 53°C for 1 min, an extension at 72°C for 1 min, and a final extension at 72°C for 8 min.

Antimicrobial susceptibility testing: Antimicrobial sensitivity test of *E. coli* isolates were performed as per the Kirby-Bauer method (Bauer *et al.* 1966) and according to guidelines provided by the Clinical and Laboratory Standards Institute of the USA (CLSI 2018). A single pure colony obtained from EMB agar was suspended in 2 ml of sterile normal saline and adjusted its turbidity to the 0.5 McFarland standards. Thereafter, sterile cotton swab was dipped into the suspension and rotated several times. The swab was swabbed over the entire surface of the Mueller Hinton Agar (MHA) plate three times to ensure equal distribution of the inoculum. A total of 15 antibiotic discs with known concentration were placed on MHA agar plate and incubated at 37°C for 24 h. The name and concentration of antibiotic discs were mentioned in Table 1. A zone of inhibition was measured, compared and interpreted as resistance, intermediate of susceptible according to the size interpretative chart furnished by the CLSI guidelines-VET0819.

### Table 1. Antimicrobial resistance pattern of ESBL *E. coli*

<table>
<thead>
<tr>
<th>Name of antibiotics</th>
<th>Resistance</th>
<th>Intermediate</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefpodoxime (10 mcg)</td>
<td>108 (100%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tetracycline (30 mcg)</td>
<td>105 (97.22%)</td>
<td>0</td>
<td>3 (2.77%)</td>
</tr>
<tr>
<td>Amoxicillin + Clavulenic acid (20/10 mcg)</td>
<td>103 (95.37%)</td>
<td>0</td>
<td>5 (4.62%)</td>
</tr>
<tr>
<td>Nalidixic Acid (30 mcg)</td>
<td>102 (94.44%)</td>
<td>2 (1.85%)</td>
<td>4 (3.70%)</td>
</tr>
<tr>
<td>Enrofloxacin (5 mcg)</td>
<td>99 (91.66%)</td>
<td>0</td>
<td>9 (8.33%)</td>
</tr>
<tr>
<td>Co-trimazaxole (1.25/23.75 mcg)</td>
<td>98 (90.74%)</td>
<td>1 (0.92%)</td>
<td>9 (8.33%)</td>
</tr>
<tr>
<td>Ampicillin (10 mcg)</td>
<td>85 (78.70%)</td>
<td>8 (7.80%)</td>
<td>15 (13.88%)</td>
</tr>
<tr>
<td>Amikacin (30 mcg)</td>
<td>62 (57.40%)</td>
<td>5 (4.62%)</td>
<td>41 (37.96%)</td>
</tr>
<tr>
<td>Chloramphenicol (30 mcg)</td>
<td>60 (55.55%)</td>
<td>15 (13.88%)</td>
<td>33 (30.55%)</td>
</tr>
<tr>
<td>Ceftriaxone (30 mcg)</td>
<td>41 (37.96%)</td>
<td>8 (7.40%)</td>
<td>59 (54.62%)</td>
</tr>
<tr>
<td>Cefoxitin + clavuxil (30/200 mcg)</td>
<td>36 (33.33%)</td>
<td>60 (55.55%)</td>
<td>12 (11.11%)</td>
</tr>
<tr>
<td>Aztreaxone (30 mcg)</td>
<td>28 (25.92%)</td>
<td>19 (17.59%)</td>
<td>61 (56.48%)</td>
</tr>
<tr>
<td>Cefoxitin (30 mcg)</td>
<td>12 (11.11%)</td>
<td>12 (11.11%)</td>
<td>84 (77.77%)</td>
</tr>
<tr>
<td>Colistin (10 mcg)</td>
<td>05 (4.62%)</td>
<td>58 (53.70%)</td>
<td>45 (41.66%)</td>
</tr>
<tr>
<td>Imipenem (10 mcg)</td>
<td>0</td>
<td>5 (4.62%)</td>
<td>103 (95.37%)</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

Isolation, identification and biochemical characterization of E. coli: The overall prevalence of E. coli from poultry fecal samples according to this study was 108 (90%), with broiler (92.85%) and layers (86%), which was similar to a study conducted in Bangladesh (Mandal et al. 2022) and in Maharashtra, India (Mahadewashwami et al. 2021). In contrast, the present study showed a higher prevalence in broilers chickens compared to research conducted in West Bengal, India, by Sarkar et al. (2013).

Confirmation of E. coli by PCR technique using ECO-1 primer: Based on the PCR technique, 108 (90%) samples out of 120 were confirmed to be positive for E. coli. Out of selected isolates, 65 (92.85%) from broilers and 43 (86%) from layers of chickens were confirmed as E. coli. (Fig.1). The sample given a positive result for the 16S rRNA gene with an amplicon size of 585 bp (Fig. 2) was correlated with the finding of Godambe et al. (2017) and Ibrahim et al. (2019).

Phenotypic confirmation of ESBL-producing E. coli: Bacteria produce beta-lactamase enzymes, making them resistant to beta-lactam antibiotics such as penicillin and 3rd generation cephalosporins. E. coli is the most prevalent ESBL generator (Andrews et al. 2018). In the current study, a total of 54 (50%) isolates with broiler 32 (29.62%) and layer 22 (20.38%) were positive for extended-spectrum beta-lactamase-producing E. coli, which is consistent with the findings of Hiroi et al. (2012), who revealed 44% ESBL-producing E. coli from a rectal sample from the slaughterhouse. The occurrence of ESBL—production in E. coli (25%) and Salmonella (27.5%) was identified in West Bengal (Chowdhury et al. 2021), which is slightly equivalent to the present finding. Kharate et al. (2019) found 54.54% and 52.04% ESBL prevalence in layers and broilers, respectively in Karnataka, India, which was higher than the current study.

Antimicrobial sensitivity pattern study: All E. coli (ESBL + non-ESBL) isolated from poultry fecal matter were found to be resistant to Cefpodoxime (Fig. 3). This result agreed with the findings of broiler chickens in Ghana (Rasmussen et al. 2015). In Indonesia, Indrawati et al. (2021) identified E. coli from poultry, drinking water, and litter and showed 89% and 94% resistance against tetracycline and nalidixic acid, respectively similar to the current study. Alvarez et al. (2022) isolated E. coli from broiler farms in Portugal and found no resistance to imipenem antibiotics, consistent with the current investigation’s findings. In the present study, higher resistance levels were also recorded for amoxicillin + clavuninc, co-trimoxazole, and ampicillin (Table 1); a similar observation was also reported by Olowe et al. (2015) from animal fecal samples in Ado Akitti, Nigeria, (Osei et al. 2021) from water supply used for poultry production in Ghana. None or lower usage of antibiotics such as imipenem, Aztreaxone, and ceftriaxone in poultry industries due to their not being accessible for usage or not being used for treatment and growth promoter, the current study revealed susceptibility to these antibiotics in E. coli isolates. Gundran et al. (2019) observed similar antibiotic susceptibility trends in the poultry sector in the Philippines and Egypt (Badr et al. 2022).

Higher resistance may develop in an organism because of readily available antimicrobial agents in the Indian market, and also lower antibiotic prices and the use of antibiotics as a growth promoter in chickens by poultry farmers leads to the development of resistance; this statement is also supported by Aworh et al. (2021) in Nigeria, who reported that developing economies have easy access to antimicrobials for both human and animal usage. Lee et al. (2004) reported the MIC range for enrofloxacin, ciprofloxacin, norfloxacin, and ofloxacin grew significantly during the same time period because of a parallel rise in the rate of gyrA mutations. A similar finding also identified high resistance to enrofloxacin in the current study.

In the present study, non-ESBL-producing isolates showed resistance against cephalosporin, tetracycline, penicillin, quinolone, and the sulfonamide class of antibiotics. While compared to ESBL and non-ESBL isolates, ESBL isolates showed more resistance than non-ESBL isolates. Similar results was recorded in Benin (Anago et al. 2015). Jocoby (2009) reported that AmpC beta-lactamase genes mediate resistance to cephalosporin, cefazolin, and penicillin class of antibiotics. The existence

![Fig. 1. Detection of E. coli using PCR method.](image)

![Fig. 2. PCR amplification of 16S rRNA gene of E. coli.](image)
of AmpC beta-lactamase genes in the chromosome of \textit{E. coli}, which were not detected in the phenotypic confirmation test, might explain the higher resistance in non-ESBL \textit{E. coli}.

In Kerala, Sebastian \textit{et al.} (2021) reported that direct contact with livestock contaminated with resistant bacteria is a common route for resistance transfer from poultry to the human population and highlighted the probability of fresh faecal matter being responsible for the dissemination of multidrug resistance \textit{E. coli} to other poultry environment samples. Similarly, the present study aimed to include those samples that would come into direct contact or go into the food chain for human consumption.

The research finding revealed that \textit{Escherichia coli} isolated from selected broiler and layer farms of healthy chickens from the Banaskantha district was resistant to at least one or more antibiotics. The study showed concern about the emergence of antimicrobial resistance, particularly a multidrug resistance pattern. Based on the current finding, we recommend that awareness should be created among poultry farmers on poultry management, prevention and control of diseases in chickens, overuse of antibiotics, and the least use of antibiotics as growth promotors. Drug selection should be used to eliminate resistant pathogens from farms. Selection of drugs is needed during treatment to avoid the spreading of multidrug resistant \textit{E. coli} pathogen in chickens and their surrounding environment.

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REFERENCES


