Acta Scientific Microbiology (ISSN: 2581-3226)

Volume 9 Issue 8 August 2026

Research Article

# Prevalence of Beta-Lactamase Genes and Efflux Activity in *Klebsiella pneumoniae* and *E. coli* Isolated from Chicken Meat Samples Collected from Dibrugar City and the Surrounding, Assam, India

**Amos O Onyango[1], Shyamalima Saikia[1], Indrani Gogoi[1], Minakshi Puzari[2] and P Chetia[3]***

*[1]Research Scholar, Department of Life Sciences, Dibrugarh University, Dibrugarh-786004, Assam, India*

*[2]Assistant Professor, Department of Life Sciences, Dibrugarh University, Dibrugarh-786004, Assam, India*

*[3]Professor, Department of Life Sciences, Dibrugarh University, Dibrugarh-786004, Assam, India*

***Corresponding Author:** P Chetia, Professor, Department of Life Sciences, Dibrugarh University, Dibrugarh-786004, Assam, India.

**DOI:** 10.31080/ASNE.2026.09.0929

**Received:** June 01, 2026

**Published:** July 29, 2026



## Abstract

The presence of β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in poultry is an important public health concern because they can be transmitted through the food chain. In this study, β-lactamase-associated resistance genes and efflux pump activity of *E. coli* and *K. pneumoniae* isolated from chicken meat in Dibrugarh and neighbouring districts of Assam, India, were investigated. In total, 210 fresh chicken meat samples were taken from retail outlets and poultry farms. Standard microbiological methods were used to identify the bacterial isolates, which were then subjected to antimicrobial susceptibility testing, phenotypic confirmation of extended-spectrum β-lactamase (ESBL) production, polymerase chain reaction (PCR) detection of blaTEM, blaSHV, blaCTX-M and blaOXA, and assessment of efflux pump activity using the ethidium bromide agar cartwheel method. Among the 513 bacterial isolates recovered, 306 (59.6%) were *E. coli* and 207 (40.4%) were *K. pneumoniae*. Overall, 228 (44.4%) isolates were resistant to third-generation cephalosporins, and Resistance was significantly higher in *E. coli* than in *K. pneumoniae* ($\chi^2 = 8.44$, $p = 0.0037$). 133 *E. coli* and 70 *K. pneumoniae* were confirmed by PCR for the presence of β-lactamase-associated genes. A total of 203 genes were detected, including blaTEM (61), blaCTX-M (61), blaSHV (53) and blaOXA (28) genes. The distribution of genes was significantly different between the two species of bacteria ($\chi^2 = 9.32$; $p = 0.025$). Activity of efflux pumps was observed in 72 (31.6%) resistant isolates, with no significant difference between *E. coli* and *K. pneumoniae* ($p = 0.126$). The results of this study showed high prevalence of β-lactamase-mediated resistance determinants and efflux-mediated Resistance among poultry-derived *E. coli* and *K. pneumoniae*, indicating that poultry meat might be a reservoir of antimicrobial Resistance. Improved antimicrobial usage, molecular surveillance, and One Health approaches are key to reducing the spread of resistant bacteria via the food supply chain.

**Keyword:** Assam; Efflux; β-Lactamase Gene; *Escherichia coli*; *Klebsiella pnuemoniae*; One Health; Poultry Meat

## Introduction

As an important pillar of economic growth, the poultry and poultry-based food industry serves as an important source of protein for a large section of the human population [1]. However, the increasing trend of contamination in the poultry food industry is becoming a serious health problem (Kashanchi., *et al*. 2017). The indiscriminate usage of antibiotics in the poultry industry is leading to an alarming upsurge in the occurrence of antibiotic resistance. Among the antibiotic resistant bacteria, *Klebsiella pneumoniae* and *Escherichia coli* have been identified as frequent offenders in poultries (Bushen., *et al*. 2021), owing to the prolonged exposure to β-lactams and fluoroquinolones used as growth promoters for livestock and poultry in animal feed [2]. Being environmental organisms and part of the standard intestinal flora of animals and birds, they are most often encountered in cross-contaminated meat, opportunistically entering the human body through the consumption of improperly handled or poorly cooked food [3]. *K. pneumoniae* and *E. coli* have been included among the top six critical, high-priority, multidrug-resistant organisms belonging to the World Health Organization priority list of antibiotic-resistant Bacteria [4]. Therefore, the detection of antibiotic resistance determinants in two such enterobacterial genera of poultry meat could provide insights into the prevalence of Resistance among them. Enterobacteriaceae members produce ESBL enzymes mainly, and these enzymes can be exchanged rapidly between bacterial species since plasmids encode them [5].

## Methodology

### Collection of samples

Samples were collected from fresh chicken meat samples from diverse retail outlets, farms, and local chicken shops in Dibrugarh District and the surrounding areas, Assam, during the time span of 6-12 months. A total of 210 fresh chicken meat samples were randomly collected in sterile, leak-proof containers. The samples were transported in a cold box to the laboratory within 3-4 h to avoid overgrowth or viability loss during examination and processing.

### Isolation and identification

25 g of the meat sample was homogenized and aseptically transferred to 225 mL of buffered peptone water for enrichment and incubated at 37 °C for 18-24 h, and then loopful of the culture broth was streaked onto freshly prepared Nutrient Agar (NA) medium. The streaked plates were incubated at 37 °C for 18-24 h. Single colonies were then selected for further identification by performing biochemical tests, including oxidase and catalase tests. The results were interpreted as per Clinical Laboratory Standard Institute (CLSI) guidelines, 2022 and Bergey's Manual of Determinative Bacteriology.

### Screening for ESBL production

Phenotypic detection of extended-spectrum β-lactamase (ESBL) production was performed for all antimicrobial susceptibility testing (AST) resistant isolates by the Double Disk Synergy Test (DDST) following CLSI (CLSI, 2022) guidelines.

Bacteria were grown overnight and then a bacterial suspension of 0.5 McFarland turbidity was prepared. Suspensions were in all cases inoculated onto Mueller-Hinton (MHA) plates using sterile cotton swabs. A disk of amoxicillin–clavulanic acid, 20/10 µg, was put in the center of the plate. The disks of cefotaxime (30 µg), ceftazidime (30 µg) and ceftriaxone (30 µg) were positioned at a distance of 20 mm (center-to-center) from the amoxicillin/clavulanic acid disk.

Aerobically plates were incubated for 18–24 h at 37°C. Isolates with an increased inhibition zone and/or the characteristic "keyhole effect" (ballooning) between any cephalosporin disk and the amoxicillin–clavulanic acid disk were deemed phenotypically positive for ESBL production [6].

A Combination Disk Test (CDT) was also carried out, with cefotaxime (30 µg) and ceftazidime (30 µg) discs alone and in combination with clavulanic acid (10 µg) to further confirm ESBL production. The CLSI (2022) criteria for ESBL positivity were interpreted as an increase in the diameter of the inhibition zone of ≥5 mm for either of the two antimicrobial agents tested in combination with clavulanic acid when compared to the antimicrobial agent alone.

Phenotypically confirmed ESBL-producing isolates were then molecularly characterized by PCR for detection of β-lactamase associated resistance genes (blaCTX-M, blaTEM, blaSHV and blaOXA) [7,8].

*Escherichia coli* ATCC 25922 (non-ESBL producer) negative controls and *K. pneumoniae* ATCC 700603 which is a ESBL producer served as the positive control.

### Genomic and plasmid DNA extraction

A single colony of the isolates from NA plates was inoculated in Nutrient broth and incubated overnight at 37 °C. The extraction of genomic and plasmid DNA was done using HiPurA genomic DNA miniprep purification Kit (HiMedia, India) and HiPurA Plasmid DNA miniprep purification Kit (HiMedia India), respectively. The purity and concentration of using a µDrop plate in a Multiskan Sky.

### Detection of β-lactamase-associated genes

The presence of ESBL-encoding associated genes was determined by PCR utilising the specified primers shown in Table 1. PCR amplification was done by following denaturation at 94°C for 10 minutes, 37 cycles of DNA at 94 °C for 30 seconds, annealing at a temperature specified for each primer, extension at 72 °C for 1 minute, and the final elongation for 7 minutes. Positive and negative controls were used for each PCR amplicon. PCR amplicons were visualized by electrophoresis in 1 % agarose gel. The presence of the $bla_{CTX-M}$, $bla_{SHV}$, or $bla_{TEM}$ and *bla*OXA gene family was determined by the bands of the correct bp size based on the previously mentioned reference. The Primers used in this procedure are listed in Table 1.

| S/N | Gene | Primer | Sequence 5'-3' | Ampli-con Size (bp) | Anneal-ing temp. | References |
|---|---|---|---|---|---|---|
| 1 | blaCTXM | CTX-F<br>CTX-R | ATGTGCAGYACCAGTAARGTKATGGC<br>TGGGTRAARTARGTSACCAGAAYSAGCGG | 592 | 55 | [5] |
| 2 | blaTEM | TEM-F<br>TEM-R | GAG TAT TCA ACA TTT CCG TG<br>TAA TCA GTG AGG CAC CTA | 678 | 55 | [9] |
| 3 | blaSHV | SHV-F<br>SHV-R | ATG CGT TAT ATT CGC CTGTGT ATT<br>AGCAGGGCGACAATCCCGCG | 628 | 55 | [10] |
| 4 | blaOXA | OXA-F<br>OXA-R | ATGAAAAACACAATACATATCAACT<br>GTGTGTTTAGAATGGTGATCGCATT | 632 | 62 | [10] |

**Table 1**: Primers for PCR amplification of β-lactamase-associated resistance genes.

Sequences, length of the expected amplicon, annealing temperatures and corresponding references for PCR-amplification of the β-lactamase-associated resistance genes (blaCTX-M, blaTEM, blaSHV and blaOXA) studied.

### Assessment of resistant bacterial strains for efflux pump activity

The Ethidium bromide (EtBr)- Agar Cartweel Method, which is Agar method based utilizes EtBr to reveal efflux pump activity in bacteria. All the isolated strains that showed Resistance to Cephalosporins were further analyzed for efflux pumps by means of the EtBr-agar method. The strains were swabbed onto Tripticase Soy Agar (TSA) plates encompassing different concentrations of EtBr and incubated for aperiod of 16 hours at 37 °C. After incubation, fluorescence was observed and recorded, and the bacterial strains which revieled lower fluorescence than the control were selected [11].

## Results

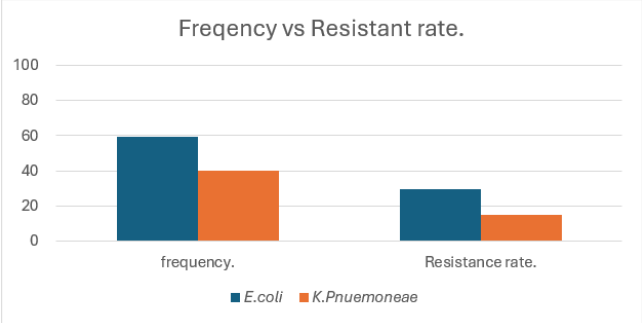
Isolation of Antibiotic-Resistant Bacteria from Chicken Meat Samples.

| Pathogen | Number of isolates | No. Resistant to Cephalosporins | Percentage of Resistance |
|---|---|---|---|
| *E.coli* | 306 | 152 | 49.6% |
| *K. pnuemoniae* | 207 | 76 | 36.7% |
| Total Isolates and %age | 513 | 228 | 44.4% |

**Table 2:** Prevalence of *E. coli* and *K. pneumoniae* that showed cephalosporin resistance in chicken meat samples.

3rd generation cephalosporin resistance of the bacterial isolates recovered from 210 chicken meat samples. Percentages are the percentage of resistant isolates among each bacterial species.

513 bacterial isolates were taken from 210 chicken meat samples; a total of 306 (59.6%) *E. coli* and 207 (40.4%) *K. pneumoniae* were isolated. In all, 228 (44.4%) isolates were resistant to the third-generation cephalosporins. Resistance was significantly higher in *E. coli* (49.6%) than in *K. pneumoniae* (36.7%) ($\chi^2 = 8.44$, $p = 0.0037$).




**Graph 1:** Frequency of resistance of cephalosporin-resistant bacterial isolates; proportion of resistance.

The graphical presentation represents the proportion of resistant isolates of each bacterial species in the constituent outbreaks of the study.

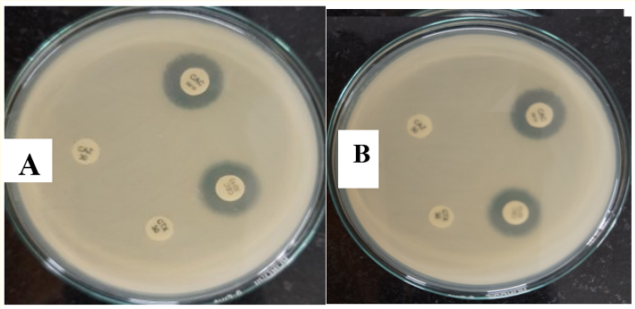



**Figure 1:** Representative phenotypic confirmation of extended-spectrum β-lactamase (ESBL) production.

Representative DDST results showing ESBL production in cephalosporin-resistant isolates. When growing on the amoxicillin–clavulanic acid disk, an enhancement of the inhibition zone (keyhole effect) is indicative of ESBL production. (A) *Klebsiella pneumoniae*. (B) *Escherichia coli*.

The 152 *E.coli* and 76 *Klebsiella* phenotypically confirmed resistant strains were further examined through PCR to detect β-lactamase-associated genes. Of which 133 *E. coli* and 70 *Klebsiella* resistant strains were genotypically confirmed to contain the named genes. encode the ESBL gene, leading to 203 genes being detected, as explained in Table 3.

| | TEM | SHV | CTX | OXA |
|---|---|---|---|---|
| *E. coli* | 43 | 40 | 31 | 16 |
| Klebsiella | 18 | 13 | 30 | 12 |
| Total genes detected | 61 | 53 | 61 | 28 |

**Table 3**: Prevalence of β-lactamase-associated resistance genes detected by PCR of *Escherichia coli* and *Klebsiella pneumoniae* resistant to cephalosporins.

203 genes encoding for β-lactamases were detected by PCR among resistant isolates. blaTEM and blaCTX-M were the most frequently detected genes (61 each), followed by blaSHV (53) and blaOXA (28).

| Gene | *E. coli* n (%) | *K. pneumoniae* n (%) | Total n (%) |
|---|---|---|---|
| blaTEM | 43 (32.3) | 18 (25.7) | 61 (30.0) |
| blaSHV | 40 (30.1) | 13 (18.6) | 53 (26.1) |
| blaCTX-M | 31 (23.3) | 30 (42.9) | 61 (30.0) |
| blaOXA | 16 (12.0) | 12 (17.1) | 28 (13.8) |
| Total gene detections | 130 (100) | 70 (100) | 203 (100) |

**Table 4**: Analysis of the distribution of β-lactamase-associated resistance genes in *Escherichia coli* and *Klebsiella pneumoniae* isolates.

$\chi^2 = 9.32$, $df = 3$, $p = 0.025$.

*E. coli* are calculated as percentages of 133 isolates with PCR positivity, while *K. pneumoniae* are based on 70 PCR-positive isolates. The total number of β-lactamase-associated gene detections was 203, and the overall percentages are based on this total.

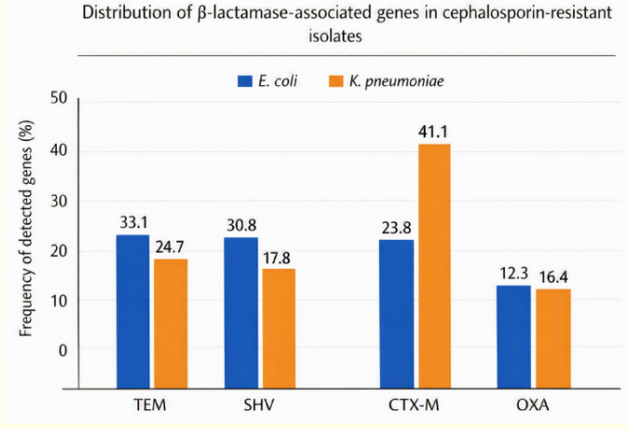



**Figure 2:** Association of β-lactamases to the strains of *Klebsiella pneumoniae* and *Escherichia coli*. Clustering and distribution of beta-lactamase genes identified in *E. coli* and *K. pneumoniae* that are resistant to cephalosporin antibiotics. The percentage frequency of blaTEM, blaSHV, blaCTX-M and blaOXA genes for each bacterial species are shown as grouped bars.

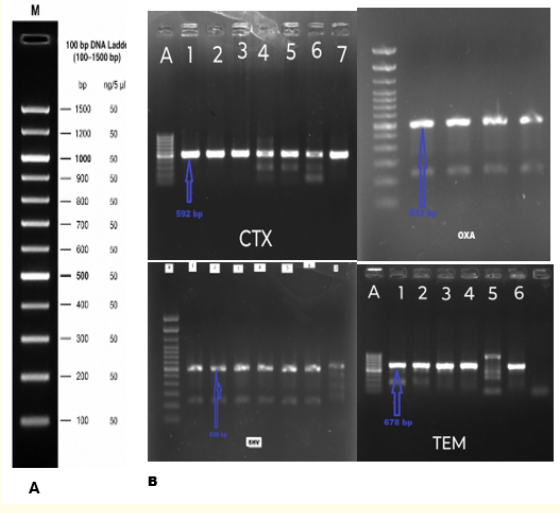



**Figure 3:** Agarose gel electrophoresis showing the PCR Amplicons of beta-lactamase-associated resistance genes in *Escherichia coli* and *Klebsiella pneumoniae* isolates from Chicken meat samples.

Representative agarose gel electrophoresis of PCR products of cephalosporin-resistant isolates. Labe M (A) 100 bp DNA ladder (100–1500 bp). (B) PCR amplicons of blaCTX-M (592 bp), blaOXA (632 bp), blaSHV (628 bp), and blaTEM (678 bp). The codes for the lane numbers are representative bacterial isolates.

## Efflux pump activity

Out of 228 Resistant strains analysed for efflux activity, 72 were indicated to have positive activity, 43 for *E. coli* and 29 for *Klebsiella*.

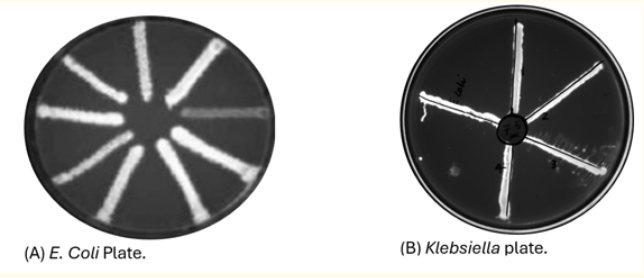



**Figure 4:** A test to detect bacterial efflux pump activity was developed using the use of ethidium bromide agar cartwheel assay.

Ethidium bromide agar cartwheel assay for efflux pumps in cephalosporin-resistant organisms. Bacterial efflux pumps actively extrude ethidium bromide, reducing the fluorescence when exposed to UV light. (A) *Escherichia coli*. (B) *Klebsiella pneumoniae*.

| Tested Organism | Number of efflux activities |
|---|---|
| *E. coli* | 43 |
| *Klebsiella* | 29 |
| Total | 72 |

**Table 5:** Efflux Pump-positive cephalosporin-resistant isolates in a distribution.

Number of cephalosporin-resistant isolates positive for efflux pump activity on the Ethidium bromide agar cartwheel assay.

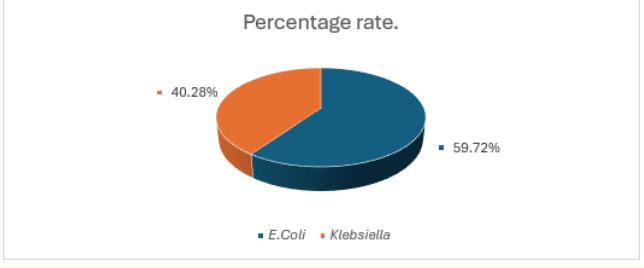



**Figure 5:** The efflux pump positive isolates as a percentage distribution of cephalosporin-resistant Escherichia coli and Klebsiella pneumoniae.

Efflux Pump-positive or total proportion of isolates on the pie chart. Of the 72 efflux-positive bacteria isolated, 43 (60.5%) were E. coli and 29 (40.3%) were K. pneumoniae.

### Statistical analysis

Categorical variables were compared using Statistical analyses based on Chi-square ($\chi^2$) test. A p-value <0.05 was deemed as statistically significant. The frequencies and percentages were used to describe the statistics. The prevalence data were presented as 95% confidence intervals (95% CI).

### Statistical Results

### Comparison of Cephalosporin Resistance prevalence between *E. coli* and *K. pneumoniae*

The bacterial strains found in the 210 samples of chicken meat were 513 in total: 306 (59.6%) *Escherichia coli* and 207 (40.4%) *Klebsiella pneumoniae*. In total, 228 (44.4%) isolates were resistant to third-generation cephalosporins. Among *E. coli,* 152 of 306 isolates (49.7%; 95% CI: 44.1–55.3%) were resistant, whereas 76 of 207 *K. pneumoniae* isolates (36.7%; 95% CI: 30.1–43.3%) were resistant. There was a significant difference in the prevalence of cephalosporin resistance between the two bacterial species ($\chi^2$ = 8.44, df = 1, p = 0.0037), with *E. coli* showing a significantly higher prevalence of Resistance to cephalosporins than *K. pneumoniae*.

### Distribution of β-Lactamase Resistance-associated genes

Of 228 cephalosporin-resistant isolates, 203 (89.0%) were confirmed using PCR to contain one or more β-lactamase-associated genes. In particular, 133 *Escherichia coli* and 70 *Klebsiella pneumoniae* strains were positive for at least one of the analysed genes (blaTEM, blaSHV, blaCTX-M, and blaOXA).

203 genes coding for β-lactamase were detected. blaTEM, with 61 (30.0%) detections, and blaCTX-M, with 61 (30.0%) detections, were the most common genes identified, followed by blaSHV with 53 (26.1%) detections and blaOXA with 28 (13.8%) detections.

Within *E. coli*, blaTEM was the most frequently detected gene (43/130; 33.1%), followed by blaSHV (40/130; 30.8%), blaCTX-M (31/130; 23.8%), and blaOXA (16/130; 12.3%).

Within *K. pneumoniae*, blaCTX-M predominated (30/73; 41.1%), followed by blaTEM (18/73; 24.7%), blaSHV (13/73; 17.8%), and blaOXA (12/73; 16.4%).

Comparing the distribution of all β-lactamase genes between *E. coli* and *K. pneumoniae* revealed a statistically significant difference (Pearson's $\chi^2$ = 9.32, df = 3, p = 0.025), indicating that the frequency of individual β-lactamase genes differs between the two bacterial species.

### Efflux pump activity

Of the 228 cephalosporin-resistant isolates, 72 (31.6%) showed positive efflux pump activity. Of these, 43 (59.7%) were *E. coli*, and 29 (40.3%) were *K. pneumoniae*. Resistant isolates were found to be efflux-active at a high frequency. *E. coli*: 43/152 (28.3%) *K. pneumoniae*: 29/76 (38.2%) Efflux activity was more prevalent between both the *K. pneumoniae* and the difference between both species is not statistically significant ($\chi^2$ = 2.34, df = 1, p = 0.126).

### Summary of statistical findings

| Comparison | Statistical Test | Test Statistic | p-value | Interpretation |
|---|---|---|---|---|
| Cephalosporin resistance between *E. coli* and *K. pneumoniae* | Pearson's Chi-square | $\chi^2$ = 8.44 (df = 1) | 0.0037 | Significant, with very high proportion of resistance found for *E. coli* as compared to *K. pneumoniae*. |
| Distribution of β-lactamase-associated genes (blaTEM, blaSHV, blaCTX-M, blaOXA) between *E. coli* and *K. pneumoniae* | Pearson's Chi-square | $\chi^2$ = 9.32 (df = 3) | 0.025 | Significant, the gene coding for β-lactamase was found at different frequencies in the two bacterial species. |
| PCR detection of β-lactamase-associated genes | Descriptive statistics | 203/228 (89.0%) PCR-positive isolates | — | In all, 133 *E. coli* and 70 *K. pneumoniae* resistant isolates contained one or more genes associated with β-lactamases. |
| Efflux pump activity between *E. coli* and *K. pneumoniae* | Pearson's Chi-square | $\chi^2$ = 2.34 (df = 1) | 0.126 | Not significant; although there appeared to be proportionately more efflux activity in *K. pneumoniae*, this difference was not statistically significant. |

**Table a**

## Discussion and Conclusion

The present study demonstrated a high prevalence of β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in chicken meat samples. Of the 210 meat samples analysed, 93.8% yielded *E. coli* and 88.6% yielded *K. pneumoniae*, indicating a substantial bacterial load in retail poultry meat. Of the 513 total isolates, 228 (44.4%) exhibited Resistance to third-generation cephalosporins, strongly suggesting β-lactamase genes production.

The resistance rate observed in *E. coli* (49.6%) was found to be higher than that of *K. pneumoniae* (36.7%). This finding collates with earlier reports, which have reported *E. coli* as the predominant ESBL reservoir in poultry [3,12]. The abundant prevalence of contamination may also suggest cross-contamination under slaughtering, processing, and sale conditions that are typically common for non-registered meat markets in several parts of India [7,8]. These results further substantiate the evidence that plasmid-mediated β-lactam resistance in foodborne Enterobacteriaceae as a whole highlights chicken meat as a major reservoir and transmission chain for multidrug-resistant (MDR) Enterobacteriaceae [10,13,14].

In addition, β-lactamase plasmids are generally accompanied by core-resistance genes that confer Resistance to aminoglycosides, tetracycline, and fluoroquinolones [15,16]; consequently, their emergence in poultry poses an important therapeutic challenge that may restrict treatment options for both veterinary and human infections [17,18].

Given this background, molecular screening was performed for the four predominant β-lactamase gene families (blaTEM, blaSHV, blaCTX-M, and blaOXA) in resistance isolates. The most frequently observed genes were blaCTX-M (61 detections), and blaTEM (61) then blaSHV (53) and blaOXA accounted for 28 detections.

The prevalence of blaCTX-M appears to be consistent with a worldwide trend, in which CTX-M types are now the most common ESBL type in Enterobacteriaceae and have replaced TEM and SHV variants [19,20]. The fact that similar CTX-M-15 and its variants are easily disseminated in both clinical and foodborne isolates indicates their widespread presence in India [9,21,22]. The nearly even prevalence of CTX-M genes in *E. coli* and *K. pneumoniae* has indicated plasmid-mediated horizontal transfer of ESBL-encoding plasmids within poultry settings.

Despite blaTEM and blaSHV ESBL types being the most common historically [16,23] found that they are carried together with CTX-M genes, pointing to the coexistence of several β-lactamase genes in the same isolate; this means an enhancement of substrate specificity and the acquisition of potentially wider Resistance. Detection of blaOXA, although less prevalent, is alarming as OXA-type β-lactamases are frequently linked to carbapenem resistance [19,24]. The occurrence of such a gene in food isolates underscores the spread of CPE (carbapenemase-producing Enterobacteriaceae) in the food chain [8,25,26]. The ethidium bromide agar cartwheel assay revealed that 72 (31.6%) of the resistant isolates exhibited efflux activity, comprising 43 *E. coli* and 29 *K. pneumoniae*. Active efflux pumps are involved in resistance mechanisms, which enable antibiotics to be actively removed from bacterial cells, resulting in a lowered intracellular concentration of the drug **[12]**. The identification of an efflux pump as a resistance mechanism indicates multiple Resistance that combines factors, which are collectively involved in the expression of ceftiofur resistance (β-lactamase production plus efflux activity) [27].

The high efflux activity expressed in *E. coli* is consistent with the possession of a higher number of β-lactamase genes, which demonstrates its adaptability to antimicrobial challenges [28]. The cooperative action of efflux pumps and β-lactamase enzymes contributes to the persistence of bacteria under the selective pressure of antibiotics, especially in poultry industries, where β-lactams and fluoroquinolones are commonly used (de Janon., *et al*. 2020).

Corresponding studies from all over India have shown similar results [29-33], with 45% of *E. coli* isolates from broiler meat being β-lactamase producers, predominantly harbouring CTX-M type genes [34] from Bangladesh reported a 38% prevalence of ESBL-producing *K. pneumoniae* in chicken meat. These localized circumstances are indicative of common problems, such as indiscriminate use of antimicrobials and poor biosecurity and surveillance [35].

The findings show that there may be identical β-lactamase gene families in both clinical and poultry isolates, which supports the one HEALTH concept [36,37]. Demonstrating that human, animal, and environmental health systems are interconnected.

### Public health and epidemiological implications

The detection of β-lactamase genes and efflux activity among foodborne isolates poses a considerable health risk [38]. Poultry is a possible source of transferring resistant bacteria or their genes into humans through either consumption or contamination during preparation [39]. These bacteria can acquire Resistance from other commensal or pathogenic species in the human gut via plasmid exchange [40].

The environmental pollution by chicken waste and antibiotic residues contributes to the persistence of the problem, resulting in an important spread of resistance genes in soil and water [41].

The findings highlight the importance of rigorous antimicrobial stewardship, improved hygiene management, and public education for controlling the transfer of AMR along the food chain [42].

## Conclusions

This study exposed a widespread incidence of ESBL-producing *E. coli* and *K. pneumoniae* isolates in poultry meat available for sale in Dibrugarh, Assam, which also exhibited efflux pump activity. The presence of multiple β-lactamase gene families, namely blaCTX-M, blaTEM, blaSHV, and blaOXA, indicates molecular diversity and possibly the mobility of β-lactamase determinants [43]. An abundance of CTX-M type β-lactamase and simultaneous efflux pump activity indicates a synthetic resistance mechanism that aids the bacterium in thriving against antibiotic stress [44]. Since resistance *E. coli* harbored such a high percentage of MRGs, it is expected that it will act as the main gene sharing pool [40,45], and because blaOXA was identified in farm animals originating from pork and pigs raised for slaughter and feeding weaning pigs, this may be an early alarm for carbapenemase-related Resistance showing up in our food supply [46,47]. The simultaneous presence of these resistance determinants underscores the severity and urgency of implementing aggressive, multisectoral interventions within a One Health context [42].

## Recommendations

This work supports the banning of non-therapeutic and growth-promoting uses of antibiotics in the poultry industry. Regulate access to prescription prophylactic antibiotics and record all use of antibiotics. Decentralization of molecular diagnostics (PCR, WGS) into the resistance surveillance scheme. Introduction of Good Hygiene Practices (GHP) and the Hazard Analysis and Critical Control Point (HACCP) system at slaughter and retail establishments. Whole genome sequencing should be used to investigate clonal relatedness and plasmid types.

## Final Remarks

This study underscores the imperative for integrated interventions to reduce AMR in the poultry industry. If not addressed, resistant bacteria , such as *E. coli* and *K. pneumoniae*, could persist in the food chain, posing risks to both animal and human health.

## Acknowledgement

We acknowledge the projects funded by DBT, Govt of India vide order nos BT/PR 16669 / 95 239 2015 and BT/PR 24808 / 95 858 2017, under which the types of equipment used in the study were procured.

## Conflict of Interest

The authors affirm no contending interests that are pertinent to the content of this article.

## Authorship Contribution Statement

Amos Oloo Onyango, Shyamalima Saikia, and Indrani Gogoi contributed equally in conceptualization, data curation, formal analysis, investigation, methodology, visualization, results validation, and writing the original draft. Minakshi Puzari reviewed and edited the work, and Pankaj Chetia supervised it. All authors read and approved the final manuscript.

## Bibliography

1. A Bushen., *et al*. "Drug- and multidrug-resistance pattern of enterobacteriaceae isolated from droppings of healthy chickens on a poultry farm in southwest Ethiopia". *Infection and Drug Resistance* 14 (2021): 2051-2058.

2. F Kashanchi., *et al*. "Antimicrobial Resistance and Molecular Epidemiology of ESBL-Producing Escherichia coli Isolated from Outpatients in Town Hospitals of Shandong Province, China". *Frontiers in Microbiology* 8 (2017): 63.

3. S Kanokudom., *et al*. "Rapid detection of extended spectrum β-lactamase producing Escherichia coli isolated from fresh pork meat and pig cecum samples using multiplex recombinase polymerase amplification and lateral flow strip analysis". (2021).

4. Kumudini Panigrah., *et al*. "Colistin Resistance Among Multi-Drug Resistant Gram-Negative Bacterial Isolates From Different Clinical Samples of ICU Patients: Prevalence and Clinical Outcomes". *Cureus* (2022).

5. M J Gharavi., *et al*. "Comprehensive study of antimicrobial susceptibility pattern and extended spectrum beta-lactamase (ESBL) prevalence in bacteria isolated from urine samples". *Scientific Reports* 11 (2021): 578, 123AD.

6. R Yulistian., *et al*. "Prevalence of Antibiotic-resistance Enterobacteriaceae strains Isolated from Chicken Meat at Traditional Markets in Surabaya, Indonesia". Article 193.012007 (2017).

7. H Saisi., *et al*. "Prevalence of CTXM, SHV, TEM AND OXA Genes among Extended-Spectrum Beta-Lactamase Producing <i>Klebsiella pneumoniae</i> from Mukuru Slum, Kenya". *Advances in Microbiology* 09.10 (2019): 853-862.

8. S Saikia., *et al*. "Co-production of metallo-β-lactamase and OXA-type β-lactamases in carbapenem-resistant Acinetobacter baumannii clinical isolates in North East India". *World Journal of Microbiology and Biotechnology* 40.6 (2024).

9. SM Khalifa., *et al*. "β-lactam resistance associated with β-lactamase production and porin alteration in clinical isolates of E. coli and K. pneumoniae". (2021).

10. H Hasman., *et al*. "b-Lactamases among extended-spectrum b-lactamase (ESBL)-resistant Salmonella from poultry, poultry products and human patients in The Netherlands". *Journal of Antimicrobial Chemotherapy* 56 (2005): 115-121.

11. H Mohanty., *et al*. "Mechanism of drug resistance in bacteria: efflux pump modulation for designing of new antibiotic enhancers". *Folia Microbiology (Praha)* 1 (2021): 3.

12. HM Sanchez., *et al*. "Antibiotic Resistance of Escherichia coli Isolated from Conventional, No Antibiotics, and Humane Family Owned Retail Broiler Chicken Meat". *Animals* 10.2217 (2020).

13. G Tellez., *et al*. "Antimicrobial Resistance in Bacterial Poultry Pathogens: A Review". Article 4 (2017): 1.

14. JG Ndukui., *et al*. "Antimicrobial Resistance Patterns of Selected <i>Enterobacteriaceae</i> Isolated from Commercial Poultry Production Systems in Kiambu County, Kenya". *Pharmacology & Pharmacy* 12.10 (2021): 219-236.

15. Z I Kimera., *et al*. "Antimicrobial use and resistance in food-producing animals and the environment: An African perspective". *BioMed Central Ltd* (2020).

16. A M Lupindu., *et al*. "Transmission of antibiotic-resistant Escherichia coli between cattle, humans and the environment in peri-urban livestock keeping communities in Morogoro, Tanzania". *Preventive Veterinary Medicine* 118.4 (2015): 477-482.

17. S A Kraemer., *et al*. "Antibiotic Pollution in the Environment: From Microbial Ecology to Public Policy". *Microorganisms* 7.180 (2019).

18. A I Gonçalves-Tenório., *et al*. "Prevalence of Pathogens in Poultry Meat: A Meta-Analysis of European Published Surveys". 7.5 (2018): 69.

19. G T Adeyanju and O Ishola. "Salmonella and Escherichia coli contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria". (2014).

20. A S Khan., *et al*. "Prevalence and serotypes of Salmonella spp. on chickens sold at retail outlets in Trinidad". *Plos One* (2018).

21. B Kowalska-Krochmal and R Dudek-Wicher. "The Minimum Inhibitory Concentration of Antibiotics: Methods, Interpretation, Clinical Relevance". *Pathogens* (2021).

22. R J Worthington and C Melander. "Overcoming Resistance to β-Lactam Antibiotics NIH Public Access". *Journal of Organic Chemistry* 78.9 (2013): 4207-4213.

23. M Touati., *et al*. "Emergence of Escherichia coli harbouring mcr-1 and mcr-3 genes in North West Algerian farmlands". *Journal of Global Antimicrobial Resistance* 21 (2020): 132-137.

24. "Microbiological quality of poultry meat: a review". *Brazilian Journal of Poultry Science Revista Brasileira de Ciência Avícola* (2004).

25. H Alkofide., *et al*. "Multidrug-resistant and extensively drug resistant enterobacteriaceae: Prevalence, treatments, and outcomes - a retrospective cohort study". *Infection and Drug Resistance* 13 (2020): 4653-4662.

26. AC Fluit., *et al*. "Molecular detection of antimicrobial resistance". (2001).

27. Kalpna D Rakholiya., *et al*. "Medicinal Plants as Alternative Sources of Therapeutics against Multidrug-Resistant Pathogenic Microorganisms Based on Their Antimicrobial Potential and Synergistic Properties". in Elsevier eBooks, Elsevier BV, (2013): 165-179.

28. Chika Ejikeugwu., *et al*. "Multiple Antibiotic Resistance, Antibiogram and Phenotypic Detection of Metallo-Beta-Lactamase (MBL) from Escherichia coli of Poultry Origin". *Journal of Applied Microbiology and Biochemistry* 1.4 (2017).

29. David Ortega-Paredes., *et al*. "Broiler Farms and Carcasses Are an Important Reservoir of Multi-Drug Resistant Escherichia coli in Ecuador". *Frontiers in Veterinary Science* 7 (2020).

30. N Neog., *et al*. "Klebsiella oxytoca and Emerging Nosocomial Infections". Springer (2021).

31. Mitul A Patel., *et al*. "Prevalence and characterisation of antimicrobial resistance pattern of ESBLproducing Escherichia coli isolated from poultry in Banaskantha district, India". *Indian Journal of Animal Sciences* 93.6 (2023).

32. Anil Shrestha., *et al*. "Multi-drug resistance and extended spectrum beta lactamase producing Gram negative bacteria from chicken meat in Bharatpur Metropolitan, Nepal". *BMC Research Notes* 10.1 (2017).

33. Shyamalima Saikia., *et al*. "Co production of metallo β lactamase and OXA type β lactamases in carbapenem resistant Acinetobacter baumannii clinical isolates in North East India". *World Journal of Microbiology and Biotechnology* (2024).

34. Badrul Hasan., *et al*. "Antimicrobial Drug-Resistant Escherichia coliin Wild Birds and Free-range Poultry, Bangladesh". *Emerging Infectious Diseases* 18.12 (2012): 2055-2058.

35. Muhammad Ali Akond., *et al*. "Frequency of drug resistant Salmonella spp. isolated from poultry samples in Bangladesh". *Stamford Journal of Microbiology* 2.1 (2013): 15-19.

36. H Badr., *et al*. "Multidrug-Resistant and Genetic Characterization of Extended-Spectrum Beta-Lactamase-Producing E. coli Recovered from Chickens and Humans in Egypt". *Animals* 12.3 (2022).

37. L Falgenhauer., *et al*. "Detection and characterization of ESBL-producing Escherichia coli from humans and poultry in Ghana". *Frontiers in Microbiology* 10 (2019).

38. A Husna., *et al*. "Extended-Spectrum β-Lactamases (ESBL): Challenges and Opportunities". Multidisciplinary Digital Publishing Institute (MDPI) (2023).

39. S Singh., *et al*. "A one health approach addressing poultry-associated antimicrobial resistance: Human, animal and environmental perspectives". Elsevier B.V (2025).

40. S Tao., *et al*. "The Spread of Antibiotic Resistance Genes In Vivo Model". Hindawi Limited (2022).

41. Y Chen., *et al*. "Antibiotic resistance gene pollution in poultry farming environments and approaches for mitigation: A system review". Elsevier Inc (2025).

42. A B Al-Tammemi., *et al*. "Tackling antimicrobial resistance in the food chain under the One Health umbrella: a systems thinking approach with a focus on Jordan". *Science in One Health* 4 (2025).

43. M Ibrahim., *et al*. "Emergence of bla TEM , bla CTX M , bla SHV and bla OXA genes in multidrug resistant Enterobacteriaceae and Acinetobacter baumannii in Saudi Arabia". *Experimental and Therapeutic Medicine* 22.6 (2021).

44. L Huang., *et al*. "Bacterial Multidrug Efflux Pumps at the Frontline of Antimicrobial Resistance: An Overview". *MDPI* (2022).

45. AS Ripanda., *et al*. "Antibiotic-resistant microbial populations in urban receiving waters and wastewaters from Tanzania". *Environmental Chemistry and Ecotoxicology* 5 (2023): 1-8.

46. E Huang., *et al*. "Carbapenem resistance in the food supply chain". Elsevier B.V (2023).

47. S Noshadi and A Khodavandi. "Expression analysis of drug-resistant gene (Blaoxa-51) in carbapenemases producing acinetobacter baumannii treated with imipenem/sulbactam combination". *Brazilian Journal of Pharmaceutical Sciences* 57 (2021).