

## Characterization of Extended-spectrum and pAmpC Beta-lactamases producing *Escherichia coli* isolated from Chicken Meat in West Bengal

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### Abstract

*Escherichia coli* is one of the pathogenic bacteria causing food-borne infections like chicken meat, which can lead to serious public health hazards. Antimicrobial resistance in *E. coli* is a serious threat to the human population nowadays. The present study aimed to detect extended-spectrum beta-lactamases (ESBLs) and AmpC beta-lactamase (ACBL)-producing *Escherichia coli* from chicken meat, from different districts of West Bengal. A total of 107 raw chicken meat samples were collected just after slaughter from different districts' local markets followed by isolation and identification of 74(69.16%) *E. coli* isolates by standard conventional and molecular methods. Among the positive isolates, 23(31.08%) were positive to ESBL production with the presence of the *bla*<sub>CTX-M</sub> gene, whereas 65(87.84%) strains were found to possess the *bla*<sub>AmpC</sub> gene. AntibioGram study of ESBL-positive *E. coli* strains revealed the sensitivity of these strains to imipenem (65.65%), gentamicin (86.96%), ampicillin/sulbactam (78.26%), and amikacin (82.61%) whereas all other antimicrobials were resistant against these pathogens.

**Keywords:** AntibioGram, ACBL, CTX-M, Chicken meat, *E. coli*, ESBL

### Introduction:

Meat production in India is growing day by day. Now, the annual meat production of India is 5.3 million metric tons which is the 5<sup>th</sup> largest in the World (DAHD, 2017). India has the world's largest livestock population which plays an important role in rural economy and livelihood. It produces 21% of global chicken meat production annually. The poultry industry is rapidly growing in India as well as in the state of West Bengal. West Bengal is the 2<sup>nd</sup> largest contributor with 640 thousand metric tons of meat production of which chicken meat production is 328 thousand metric tons (DAHD, 2017). But chicken meat can easily get spoilt with bacterial spoilage due to faulty handling, improper storage, and poor management of the birds (Dierikx et al., 2010).

Antimicrobial resistance (AMR) is increasing day by day. With the increased consumption of antibiotics, in the last few decades leading to a rise in their resistance among microbial populations. The incidence of extended-spectrum beta-lactamases (ESBLs)-producing *Escherichia coli* in food is quite significantly increasing nowadays all around the World. ESBL production in bacteria is governed by the presence of *bla*<sub>CTX-M</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>TEM</sub> genes which are easily transferred from one bacterium to another spreading the antimicrobial resistance. Among these resistance genes, the *bla*<sub>CTX-M</sub>

gene is the most common gene associated with ESBL positivity in *E. coli* (Dierikx et al., 2010). This drug-resistant pathogen can create a major problem during their treatment forcing the clinicians to use newer and newer antibiotics (Tenover et al., 1999; Olesen et al., 2004). These antimicrobial resistance genes of *E. coli* are easily transferrable to other pathogens conferring them resistance. The AmpC beta-lactamase (ACBL) is the first bacterial enzyme reported to destroy penicillin in Gram-negative bacteria like *Escherichia coli*. ACBL encoding gene *bla*<sub>AmpC</sub> is found in transmissible plasmids and also in bacterial chromosomes (Reich et al., 2013). ACBL-producing *E. coli* strains are resistant to broad-spectrum cephalosporins but their resistance patterns are less expressed *in-vitro* than that of the ESBLs (Jacoby, 2009).

Common people consider chicken meat as a very popular source of animal protein worldwide. ESBL-producing *E. coli* is frequently reported from chicken samples worldwide and may be pathogenic to humans causing urinary tract infections, septicemia, meningitis, etc. (Grami et al., 2013; Nandanwar et al., 2014). Most of the countries are using a large quantity of different antimicrobials to raise poultry which are also used in human treatments. Indiscriminate use of such essential antimicrobials in animal production is likely to accelerate the resistance development of pathogens, as well as commensal organisms like *E. coli*. This would result in

treatment failures and economic loss and could act as a source of the gene pool for transmission to humans (Castanon, 2007). In addition, there are human health concerns about the presence of antimicrobial residues in meat, eggs, and other animal products (Sahoo et al., 2010; Darwish et al., 2013).

Identification of potential MDR pathogenic bacteria is essential towards the development of better managerial procedures. With this background, the present research has aimed at the detection and characterization of ESBL and ACBL-producing *E. coli* from raw chicken meat collected from different local markets of West Bengal and followed by an *in-vitro* antibiogram to assess their resistance patterns.

## Materials and Methods:

**Sample collection:** A total of 107 chicken meat samples were collected at the time of slaughter from local markets of Paschim Medinipur, Nadia, and Hooghly district of West Bengal during the period of August to December 2022. Ten grams of fresh meat samples were aseptically collected in individual vials and transported (under ice cover) to the laboratory. Samples were enriched on the date of receiving in the laboratory.

**Bacteriological isolation and characterization:** A 10% homogenized suspension of each meat sample was prepared in sterile normal saline and streaked onto MacConkey's agar (Hi-Media, India) and then onto EMB agar (Hi-Media, India) plates followed by incubation overnight at 37°C. The chocolate colour colonies with a 'metallic sheen' were picked up for further morphological (by Gram's staining) and biochemical characterization (Carter and Wise, 2004; Quinn et al., 2011). One tentative *E. coli* isolate from each sample was taken in this study.

**Confirmation of *Escherichia coli* by PCR:** All the tentative *E. coli* isolates were confirmed by detection of the 16S rRNA gene specific for this genus, following the protocol of Wang et al., 1996 (Table 1).

**Phenotypic detection of ESBL in *E. coli* strains:** Phenotypic detection of ESBL activity of the *E. coli* isolates was done by double disc diffusion assay (Bauer et al., 1966) using both cefotaxime (30 µg) and ceftazidime disks (30 µg) and their clavulanate (10 µg) discs as per CLSI method of Patel et al. (2015). An increase of zone diameter (>5 mm) in each clavulanate disk than the single drug disk is treated as phenotypical confirmation of the ESBL activity.

**Molecular detection of ESBL positivity:** All the *E. coli* isolates were screened for ESBL positivity by the detection of the *bla*<sub>CTX-M</sub> gene by PCR assay as per the protocol of Weill et al. (2004) (Table 1). In this method, 5 µl bacterial DNA templates, 50 pmol of each primer,

200 mM deoxynucleoside triphosphate, 1 U Taq DNA polymerase (Promega, USA), 2 mM MgCl<sub>2</sub>, and 10% dimethyl sulfoxide (DMSO) was added in a 25 µl reaction mixture and subjected to amplification with following PCR conditions - 10 mins of initial denaturation at 94°C followed by 30s of denaturation at 94°C, 30s of annealing at 53°C and 1 min of extension at 72°C for 35 cycles and 10 mins of final extension at 72°C. The amplified product was visualized by gel documentation system (UVP, UK) after electrophoresis in 1.5% (w/v) agarose (SRL, India) gel containing ethidium bromide (0.5 µg/ml) (SRL, India). In this study, an *Escherichia coli* serotype O2, maintained in the departmental laboratory was used as the positive control.

**Phenotypic detection of ACBL Production in *Escherichia coli* isolates:** *In-vitro* ACBL activity of all *Escherichia coli* isolates was examined following the protocol of Tan et al. (2009) by cefoxitin–cloxacillin double-disc synergy (CC-DDS) test.

**PCR detection of *AmpC* gene in *E. coli* strains:** All the *E. coli* strains were examined for the presence of the *AmpC* gene by PCR following the protocol of Faria et al., 2002 (Table 1). In this method, the total reaction volume was 25 µl containing 5 µl of bacterial DNA template, 100 pmol of each primer, 200 mM of each dNTP, 2 mM MgCl<sub>2</sub>, and 10% DMSO. The PCR mixture was subjected to an initial denaturation step of 5 mins at 94°C; followed by 30 cycles of amplification consisting of 30s of denaturation at 94°C, 30s of annealing at 57°C, 1 min of elongation at 72°C and 10 mins of final extension at 72°C. The PCR product was electrophoresed in 1.5% (w/v) agarose (SRL, India) gel containing ethidium bromide (0.5 µg/ml) (SRL, India) and the gel was visualized in a gel documentation system (UVP, UK).

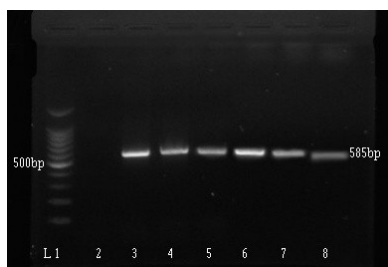
**Antibiogram of ESBL-positive *E. Colis* strains:** *In-vitro* antibiotic sensitivity of the ESBL gene-positive *E. coli* isolates was examined using 10 commonly used antimicrobials viz. ampicillin, amikacin, ampicillin/clavulanic acid, ampicillin/sulbactam, cefotaxime, ceftriaxone, ceftazidime, gentamicin, imipenem, and norfloxacin by disc diffusion method (Bauer et al., 1966). Standard antibiotic discs (Hi-Media, India) were used as the source of antibiotics. The inhibition zone diameters were interpreted following the standard chart (Patel et al., 2015).

**Table 1: Details of Target Genes and their Primers used in this Study**

Gene	Primer sequence	Size	Reference
<i>E. coli</i> 16S <i>rRNA</i>	ECO-1 F 5'GACCTCGGTTTAG TTCACAGA3' ECO-2 R 5'CACACGCTGACG CTGACCA3'	585 bp	Wang et al., 1996
<i>bla</i> <sub>CTX-M</sub> consensus (ESBL)	CTX-M F 5' CCATGTGCAGCACC AGTAA 3' CTX-M R 5' CGCAATATCCTTGG TGGTG 3'	540 bp	Weill et al., 2004
<i>bla</i> <sub>AmpC</sub> (ACBL)	AmpC F 5'CCCCGCTTATAGA GCAACAA3' AmpC R 5'TCAATGGTTCGACT TCACACC3'	634 bp	Feria et al., 2002

## Results and Discussion:

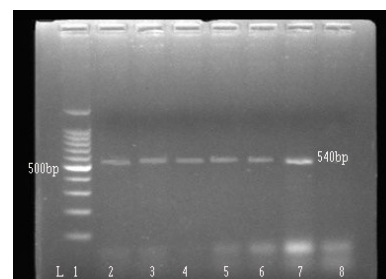
Approximately 74 (69.16%) chicken meat samples were tested to be positive for *Escherichia coli* in this study. All the isolates of *E. coli* were characterized by pinkish colonies on Mac Conkey's agar, characteristic 'metallic sheen' on EMB agar plates, Gram's negative rods, and positive reaction to the indole test. All the isolates were detected to possess the 16S *rRNA* gene (Figure 1) sterile EMB agar (Hi-Media, India) plates and thus confirmed to be *E. coli* (Carter and Wise, 2004; Quinn et al., 2011; Samanta, 2013). This study identified a very high prevalence (78.86%) of *Escherichia coli* in poultry meat, which matches with earlier works of Reich et al. (2013), Maciucă et al. (2015), and Klimiene et al. (2018) who reported 45%, 54% and 92% *E. coli* prevalence in chicken meat from different countries showing the significant presence of the pathogen in the food chain (Dierikx et al., 2010). Samples from the Hooghly district were mostly infected (79.17%) followed by Paschim Medinipur and Nadia (Table 2).



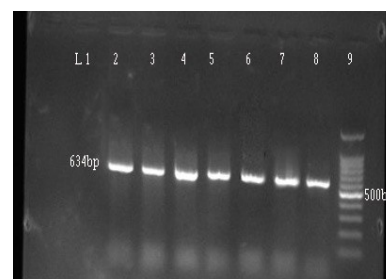
**Figure 1: Detection of the 16S *rRNA* gene (585bp) in *E. coli* isolates by PCR (Lane 1: 100bp ladder, L2:**

**uninoculated negative control, L3: positive control, L4-8: test samples)**

A total of 18(24.32%) *E. coli* isolates were detected to be phenotypical ESBL producers; whereas 23(31.08%) isolates were found to possess the *bla*<sub>CTX-M</sub> gene (Figure 2) by PCR. Again 57(77.03%) strains were positive to ACBL production and a total of 65(87.84%) *E. coli* isolates were confirmed to possess the *bla*<sub>AmpC</sub> gene genotypically (Figure 3, Table 2). Seventeen *E. coli* strains had both the genes (Table 2). Such higher ESBL positivity in poultry *E. coli* isolates has also been reported from different countries by Maamar et al. (2016), Tekiner and Ozpinar (2016), Casella et al. (2017) and Klimiene et al. (2018). The gene *bla*<sub>CTX-M</sub> is the most common and dominant gene among all ESBL genes (Feria et al., 2002; Samanta, 2013). The present study also confirms the earlier reports and identifies it as a potential threat to even human health (Dierikx et al., 2013). The *AmpC* beta-lactamase enzyme is also highly prevalent (87%) among the *E. coli* strains, and the prevalence rate was higher than the observations of Casella et al., 2017 (3.9%) and Van et al., 2008 (23.7%).



**Figure 2: Molecular detection of the *bla*<sub>CTX-M</sub> gene (540bp) in *E. coli* isolates by PCR (Lane1: 100bp ladder, L2-6: test samples, L7: positive control, L8: uninoculated negative control)**



**Figure 3: PCR Detection of the *bla*<sub>AmpC</sub> gene (634bp) in *E. coli* isolates by PCR (Lane 1: uninoculated negative control, L2: positive control, L3-8: test samples, L9: 100bp ladder).**

*In-vitro* antibiogram of the ESBL-positive *E. coli* isolates revealed high-level resistance (74-100%) to ampicillin,

ceftriaxone, cefotaxime, ceftazidime, ampicillin-clavulanic acid, and norfloxacin (Table 3). In contrast, isolates were sensitive to drugs like amikacin, gentamicin, imipenem, and ampicillin-sulbactam. Such high-level drug resistance by the ESBL-positive *E. coli* isolates was also reported by Van et al. (2008), Reich et al. (2013), Beninati et al. (2015) and Maamar et al. (2016). Again, Tekiner and Ozpinar (2016) reported that *E. coli* from raw chicken meat were resistant to cefotaxime (62.1%), ceftazidime (55.2%), cefoperazone (51.7%) and cloxacillin (20.6%). Van et al. (2008) also reported multidrug resistance among poultry meat *E. coli*, which were resistant to tetracycline (77.8%), ampicillin

(50.5%), gentamicin (24.2%) and norfloxacin (17.2%) although few other studies indicated that ESBL positive *E. coli* strains are sensitive to few beta-lactams and aminoglycosides like amikacin, imipenem, ampicillin-sulbactam, and gentamicin (Tekiner and Ozpinar, 2016; Castillo et al., 2018). The rapid increase in the development and spread of antimicrobial resistance (AMR) is a matter of serious concern (Van et al., 2008; Ryu et al., 2012).

**Table 2: Detection and distributions of beta-lactamases producing genes among *E. coli* isolates from chicken meat in West Bengal**

Name of the Districts	No. of meat samples screened	No. of <i>E. coli</i> strains Isolated (%)	ESBL positivity in <i>E. coli</i> strains (%)	ACBL Positivity in <i>E. coli</i> strains (%)	Gene distribution in positive <i>E. coli</i> strains		
					<i>bla</i> <sub>CTX-M</sub> only	<i>bla</i> <sub>AmpC</sub> only	<i>bla</i> <sub>CTX-M</sub> + <i>bla</i> <sub>AmpC</sub>
Paschim Medinipur	37	26 (70.27)	6 (23.07)	21 (80.77)	2	18	5
Nadia	46	29 (63.04)	7 (24.14)	23 (79.31)	3	19	6
Hooghly	24	19 (79.17)	5 (26.31)	13 (68.42)	1	11	6
Total	107	74 (69.16)	18 (24.32)	57 (77.03)	6	48	17

**Table 3: Resistance pattern of 23 ESBL-positive *E. coli* isolates obtained from chicken meat in West Bengal**

Sl. No.	Antimicrobials (Conc. in µg)	Isolates sensitive		Isolates intermediately sensitive		Isolates resistant	
		No.	%	No.	%	No.	%
1.	Amikacin (AK - 30)	19	82.61	4	17.39	0	0
2.	Ampicillin / Clavulanic acid (AMC - 30)	0	0	1	4.35	22	95.65
3.	Ceftriaxone (CTR 30)	0	0	0	0	23	100
4.	Ampicillin/Sulbactam (A/S - 10/10 mcg)	18	78.26	5	21.74	0	0
5.	Ampicillin (AM - 10)	0	0	0	0	23	100
6.	Ceftazidime (CAZ - 30)	0	0	1	4.35	22	95.65
7.	Imipenem (IPM - 10)	22	95.65	1	4.35	0	0
8.	Gentamicin (GEN - 10)	20	86.96	3	13.04	0	0
9.	Norfloxacin (NX - 10)	0	0	6	26.09	17	73.91
10.	Cefotaxime (CTX - 30)	0	0	1	4.35	22	95.65

## Conclusion:

It can be concluded that approximately 69% of the chicken meat samples screened in this study, were found to be positive for *E. coli* strains. About 31% and 87% of these bacteria were positive for ESBL and ACBL production which are quite significant data. The ESBL-positive stains were resistant to most commonly used antimicrobials other than amikacin, imipenem, gentamicin, and ampicillin/sulbactam. These drug-resistant *Escherichia coli* strain can become a serious concern for causing animal and human health.

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## Conflict of Interest:

No competing interest exists among the authors.

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