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Short Communication

Detection of ESBL-producing *Escherichia coli* in milk and eggs from Kolkata, India

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Abstract

Animal-origin foods such as milk and eggs are important sources of nutrients but are also susceptible to microbial contamination, posing significant public health risks. This study investigated the occurrence of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* in milk and eggs collected from in and around Kolkata. A total of 35 samples collected using a random sampling approach from selected dairy farms and backyard poultry units collected randomly, comprising raw milk (n=9) and eggs (n=26; 13 eggshell swabs and 13 yolk samples), were analysed using cultural, phenotypic and molecular methods. Presumptive *E. coli* was detected in 48.57% of samples, with higher prevalence in milk (88.89%) compared to eggs (34.62%). Molecular confirmation using 16S rRNA PCR identified 9 isolates as *E. coli*. Phenotypic screening revealed ESBL production in 44.44% of confirmed isolates, including 40% from milk and 50% from egg samples. Antimicrobial susceptibility testing demonstrated multidrug resistance, with complete resistance to ampicillin and ticarcillin/clavulanic acid and high resistance to ceftriaxone, oxytetracycline, and sulphadiazine. Genotypic analysis detected bla_{CTX-M} and bla_{SHV} genes among selected isolates, with both genes identified in eggshell-derived isolates, indicating a predominance of external contamination. The discrepancy between phenotypic and genotypic findings suggests the presence of additional resistance determinants. The study highlights the role of milk and eggs as potential sources of antimicrobial-resistant *E. coli* and emphasises the need for improved hygiene, prudent antimicrobial use, and continuous surveillance under a One Health framework.

Keywords: Antimicrobial resistance, Eggs, ESBL, *Escherichia coli,* Milk

Highlights

- Detection of ESBL-producing *E. coli* in milk and egg samples from Kolkata
- Occurrence of 44.44% of confirmed isolates exhibited phenotypic ESBL production
- Multidrug resistance observed with high β-lactam resistance

Milk and eggs are widely consumed animal-origin foods that serve as rich sources of essential nutrients; however, their high moisture content and nutrient composition make them susceptible to microbial contamination (Sharan et al., 2023). Contamination may arise from poor hygiene, environmental exposure, infected animals or improper handling during production and storage, posing significant public health risks. Among the microorganisms associated with such contamination, *Escherichia coli* is frequently detected and is widely recognized as an indicator of faecal contamination in food and environmental samples (Odonkor & Ampofo, 2013; Devane et al., 2020). Certain pathogenic strains, particularly Shiga toxin-producing *E. coli* (STEC) such as O157:H7, are associated with severe illnesses, including haemolytic uremic syndrome, and are commonly transmitted through contaminated animal-derived foods (Gambushe et al., 2022; World Health Organisation [WHO], 2018). Recent studies have highlighted increasing antimicrobial resistance in foodborne pathogens, particularly in developing countries (WHO, 2023). The growing emergence of antimicrobial resistance (AMR), particularly extended-spectrum β-lactamase (ESBL)-producing *E. coli*, further heightens this risk by restricting available treatment options. However, despite the significance of this issue, evidence on the occurrence of ESBL-producing *E. coli* in foods of animal origin within this region remains limited. Accordingly, the present study was designed to assess the presence of ESBL-producing *E. coli* in milk and eggs collected from Kolkata and its surrounding areas.

Samples comprising raw cow milk (n=9) and eggs (n=26; 13 shell swabs and 13 yolk samples) were collected randomly and aseptically from local dairy farms (n=2) and backyard poultry units (n=3) in the vicinity of Kolkata. Farms were selected based on the

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willingness of the farmers to participate in the study and distance of the farms from the city. Samples were transported under chilled conditions and processed within 24 hours. The collected samples were inoculated into nutrient broth (HiMedia, India) and incubated at 37°C for 18–24 hours. A loopful of culture was streaked onto MacConkey agar (HiMedia, India), and lactose-fermenting colonies were subcultured on eosin methylene blue (EMB) agar (HiMedia, India). Colonies exhibiting a characteristic green metallic sheen were presumptively identified as *E. coli* and maintained on nutrient agar slants, with long-term storage in brain heart infusion (BHI) broth (HiMedia, India) supplemented with 20% glycerol at –20°C. Morphological identification was performed by Gram staining following standard procedures (Merchant & Packer, 1967).

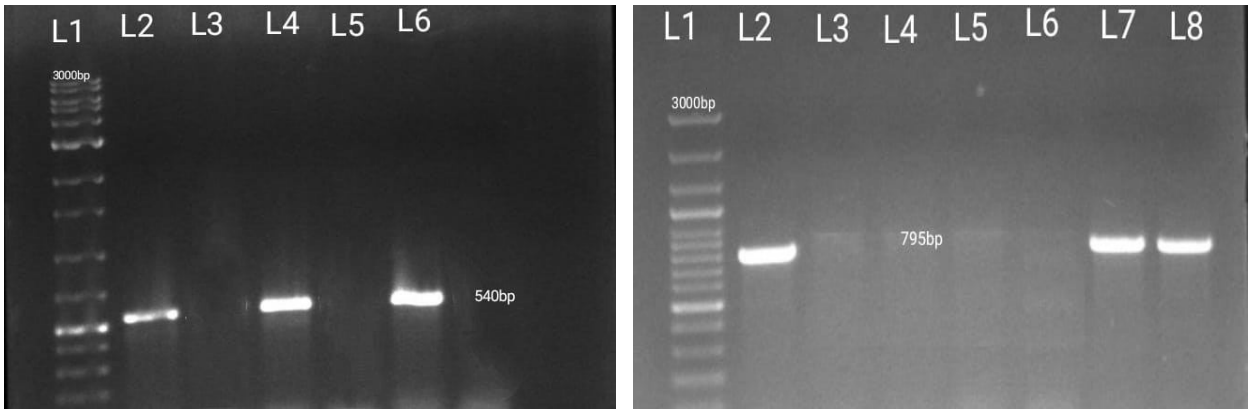
Genomic DNA was extracted using the boiling and centrifugation method as described by Salehi et al. (2005) with slight modifications (boiling for 15 minutes in a hot water bath and centrifugation at 6500 RPM). Molecular confirmation of *E. coli* isolates was carried out by PCR targeting the 16S rRNA gene, following the protocol of Wang and Wang (1996). However, it is acknowledged that 16S rRNA may not provide species-level specificity for *E. coli*, which is a limitation of the study. PCR amplification was performed using standard reagents (Promega, USA), and amplicons were resolved by electrophoresis in 1.5% agarose gel (HiMedia, India) stained with ethidium bromide and visualized under a gel documentation system.

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method (Bauer et al., 1966) on Mueller–Hinton agar (HiMedia, India) following Clinical and Laboratory Standards Institute guidelines (Clinical and Laboratory Standards Institute [CLSI], 2017) using commercially available antibiotic discs (HiMedia, India). Isolates resistant to at least one agent in three or more antimicrobial classes were considered multidrug-resistant. Phenotypic detection of extended-spectrum β-lactamase (ESBL) production was carried out by the double-disk synergy test using cefotaxime and ceftazidime, with and without clavulanic acid, following CLSI guidelines (CLSI, 2023) and the method described by Drieux et al. (2008). Further molecular characterization of ESBL-producing isolates was performed by PCR targeting bla_{CTX-M} and bla_{SHV} genes following previously described protocols (Cao et al., 2002; Weill et al., 2004). ESBL-producing *E. coli* reference strains used as positive controls were obtained from the Department of Veterinary Microbiology, West Bengal University of Animal and Fishery Sciences, Kolkata.

A total of 35 samples comprising raw milk (n=9) and eggs (n=26; 13 eggshell swabs and 13 egg yolk samples) were analysed for the presence of *E. coli*. Based on cultural characteristics on MacConkey agar and eosin methylene blue (EMB) agar, presumptive *E. coli* was detected in 17 samples (48.57%), including 8/9 (88.89%) milk samples and 9/26 (34.62%) egg samples. Among egg samples, isolates were predominantly recovered from eggshell swabs compared to yolk samples, indicating higher levels of external contamination. All isolates exhibited characteristic lactose-fermenting colonies on MacConkey agar and green metallic sheen on EMB agar, and were identified as Gram-negative, short rod-shaped bacteria. Molecular confirmation using PCR targeting the 16S rRNA gene identified a total of 9 *E. coli* isolates, comprising 5 isolates from milk samples and 4 isolates from egg samples. The distribution of *E. coli* isolation, molecular confirmation, and ESBL detection among milk and egg samples is summarized in Table 1. Earlier study also confirmed a smaller number of *E. coli* isolates from sheep and goat faecal samples by 16S rRNA gene specific PCR in comparison to conventional biochemical tests, as phenotypical tests sometimes generate false-positive results and identify other bacteria as *E. coli* (Kumar et al., 2019).

Table 1. Distribution and isolation of *E. coli* from milk and egg samples

Sample type	No. of samples	Presumptive *E. coli* positive	Confirmed by16S rRNA PCR	ESBL-positive (phenotypic)	bla_{CTX-M}	bla_{SHV}
Milk	9	8 (88.89%)	5 (55.56%)	2 (40.00%)	1 (20.00%)	2 (40.00%)
Eggs (total)	26	9 (34.62%)	4 (15.38%)	2 (50.00%)	1 (25.00%)	1 (25.00%)
Total	35	17 (48.57%)	9 (25.71%)	4 (44.44%)	2 (22.22%)	3 (33.33%)



A B

Fig. 1. Agarose gel electrophoresis showing amplification of ESBL-associated genes in *E. coli* isolates. A) Detection of bla_{CTX-M} gene (540 bp); B) Detection of bla_{SHV} gene (795 bp). L1: 100 bp DNA ladder; L2: Positive control; L3-L6 (A) and L3-L8 (B): test isolates showing presence or absence of target genes

Phenotypic detection of extended-spectrum β-lactamase (ESBL) production using the double-disk synergy test revealed ESBL activity in 4 out of 9 (4/9, 44.44%) confirmed *E. coli* isolates. Among these, 2/5 (40%) isolates from milk samples and 2/4 (50%) isolates from egg samples demonstrated ESBL production. Antimicrobial susceptibility testing of all *E. coli* isolates (n=9) revealed a multidrug-resistant profile. Complete resistance (100%) was observed against ampicillin and ticarcillin/clavulanic acid and sulphadiazine, while high resistance was also observed against ceftriaxone and oxytetracycline. In contrast, relatively higher sensitivity was observed for azithromycin and chloramphenicol, while moderate sensitivity was noted for amikacin, levofloxacin, and streptomycin.

Genotypic characterization revealed the presence of ESBL-associated genes among selected isolates. In milk-derived isolates, the bla_{SHV} gene was detected in 2 isolates (40%), while bla_{CTX-M} was detected in 1 isolate (20%). Among egg-derived isolates, bla_{CTX-M} and bla_{SHV} genes were each detected in one isolate, both originating from eggshell swabs, indicating that ESBL determinants were primarily associated with external egg contamination rather than internal contents. The PCR amplification profiles of the ESBL genes are presented in Fig. 1 (A & B).

Animal-origin foods such as milk and eggs are recognized as important reservoirs of *E. coli*, including antimicrobial-resistant strains, due to continuous exposure of food-producing animals to environmental microbes, farm management practices, and antimicrobial use. The present study demonstrated the occurrence of *E. coli* in both milk (88.89%) and egg samples (34.62%), indicating broad contamination across dairy and poultry systems. However, the relatively small sample size limits the generalization of these findings. Similar findings have been reported in previous studies, where the prevalence of *E. coli* in dairy products ranged from 21% to 77% (Ombarak et al., 2016), and approximately 30% in raw milk samples (Ranjbar et al., 2018), supporting the observed contamination levels in the current investigation.

The higher recovery of *E. coli* from eggshell samples compared to yolk samples suggests that contamination is predominantly external, likely originating from faecal matter, litter, or handling practices. This observation is consistent with earlier reports indicating that eggshell contamination serves as a major route of bacterial transmission, whereas internal contamination is relatively less frequent but still of concern under poor hygienic conditions (Gantois et al., 2009).

A key finding of this study was the detection of extended-spectrum β-lactamase (ESBL)-producing *E. coli* in both milk and egg samples. Phenotypic ESBL production was observed in 44.44% of confirmed isolates, highlighting the presence of β-lactamase-mediated resistance in animal-origin foods. Comparable studies have reported increasing prevalence of ESBL-producing *E. coli* in food-producing animals and their products, reflecting the global rise of antimicrobial resistance in the food chain, including India (Madec et al., 2017; Taskeen et al., 2025).

Genotypic analysis demonstrated the presence of bla_{CTX-M} and bla_{SHV} genes among selected isolates, with bla_{SHV} being more frequently detected in milk isolates, while both genes were identified in eggshell-derived isolates. The predominance of CTX-M and SHV-type

Table 2. Antimicrobial susceptibility pattern of *E. coli* isolates (n=9) from milk and egg samples

Antibiotics used	Milk (n=5)			Egg (n=4)		
	S(%)	I(%)	R(%)	S(%)	I(%)	R(%)
Azithromycin (15 µg)	5/5 (100.00)	0/5 (0.00)	0/5 (0.00)	3/4 (75.00)	0/2 (0.00)	1/4 (25.00)
Amikacin (30 µg)	2/5 (40.00)	2/5 (40.00)	1/5 (20.00)	2/4 (50.00)	2/4 (50.00)	0/4 (0.00)
Chloramphenicol (25 µg)	4/5 (80.00)	0/5 (0.00)	1/5 (20.00)	4/4 (100.00)	0/4 (0.00)	0/4 (0.00)
Ceftriaxone (30 µg)	2/5 (40.00)	0/5 (0.00)	3/5 (60.00)	3/4 (75.00)	1/4 (25.00)	0/4 (0.00)
Oxytetracycline (30 µg)	2/5 (40.00)	0/5 (0.00)	3/5 (60.00)	0/4 (0.00)	0/4 (0.00)	4/4 (100.00)
Levofloxacin (5 µg)	2/5 (40.00)	1/5 (20.00)	2/5 (40.00)	3/4 (75.00)	1/4 (25.00)	0/4 (0.00)
Streptomycin (10 µg)	2/5 (40.00)	2/5 (40.00)	1/5 (20.00)	3/4 (75.00)	1/4 (25.00)	0/4 (0.00)
Sulphadiazine (100 µg)	0/5 (0.00)	0/5 (0.00)	5/5 (100.00)	3/4 (75.00)	0/4 (0.00)	1/4 (25.00)
Ampicillin (10 µg)	0/5 (0.00)	0/5 (0.00)	5/5 (100.00)	0/4 (0.00)	0/4 (0.00)	4/4 (100.00)
Ticarcillin/Clavulanic acid (75/10 µg)	0/5 (0.00)	0/5 (0.00)	5/5 (100.00)	0/4 (0.00)	0/4 (0.00)	4/4 (100.00)

ESBLs has been widely documented in *Enterobacteriaceae* and is considered a major driver of resistance to third-generation cephalosporins (Bevan et al., 2017). However, the relatively lower detection of ESBL genes compared to phenotypic resistance observed in this study suggests the possible involvement of other resistance determinants, such as bla_{TEM}, *bla*AmpC or less commonly screened ESBL variants, as well as limitations in primer specificity or gene expression levels.

Antimicrobial susceptibility profiling further revealed a multidrug-resistant (MDR) pattern among isolates, with complete resistance to ampicillin and ticarcillin/clavulanic acid and high resistance to ceftriaxone and oxytetracycline. These results align with previous studies that have underscored the pervasive resistance to β-lactam antibiotics and tetracyclines in *E. coli* from animal sources. This resistance has been largely ascribed to the overuse of these antibiotics in veterinary medicine (Van Boeckel et al., 2015). In contrast, azithromycin showed consistently high sensitivity, whereas chloramphenicol exhibited variable sensitivity between milk- and egg-derived isolates. The detailed antimicrobial susceptibility pattern of ESBL-producing *E. coli* isolates from milk and egg samples is presented in Table 2.

The detection of ESBL-producing and multidrug-resistant *E. coli* in commonly consumed animal-origin foods underscores a significant public health concern. These organisms can be transmitted to humans through the food chain, contributing to treatment failures and increased disease burden. The findings of this study align with the One Health perspective, emphasizing the interconnectedness of animal, human, and environmental health in the dissemination of antimicrobial resistance.

The present study underscores the critical role of foods derived from animals, especially raw milk and eggs, as significant carriers of *E. coli*, including extended-spectrum β-lactamase (ESBL)-producing and multidrug-resistant strains. The detection of *E. coli* in a large number of milk specimens and its existence in both eggshell and yolk samples suggests possible contamination at various points during production and handling processes. The occurrence of phenotypic ESBL production, along with the identification of bla_{CTX-M} and bla_{SHV} genes, highlights the circulation of clinically relevant resistance determinants within the food chain.

The predominance of contamination in eggshell samples suggests that external contamination plays a key role in bacterial transmission. Moreover, the presence of multidrug resistance patterns underscores the limited treatment options when dealing with these

particular bacterial strains. Although genotypic detection was lower than phenotypic expression, this discrepancy could signal the existence of additional resistance mechanisms that weren't explored in this study.

The findings of this research underscore the critical importance of addressing the issue of antimicrobial-resistant *E. coli* in commonly consumed foods derived from animals. Although the sample size was limited in the present study, the findings are indicative for a pressing need to strengthen hygiene practices and promote rational usage of antimicrobials in livestock rearing. Furthermore, it sheds light on the necessity of continuous surveillance within the context of the One Health framework. This approach, which encompasses human, animal, and environmental health with more sampling in future, can be instrumental in managing the ongoing public health challenge posed by antimicrobial resistance.

Conflict of interest: The authors have no conflict of interest.

Author's contribution: SB, AA: Conducted the study with equal contribution; AP, CM, DJS: Supervision and technical help; DJS: Primary manuscript preparation; IS: Supervision and critical evaluation of the manuscript

Data availability statement: All relevant data have been presented within the manuscript. The corresponding author is willing to provide the raw data upon reasonable request.

Ethical statement: No live animal or human subjects were used in the study hence ethical statement is not applicable.

Use of artificial intelligence tools: No such tools were used for preparation of manuscript

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