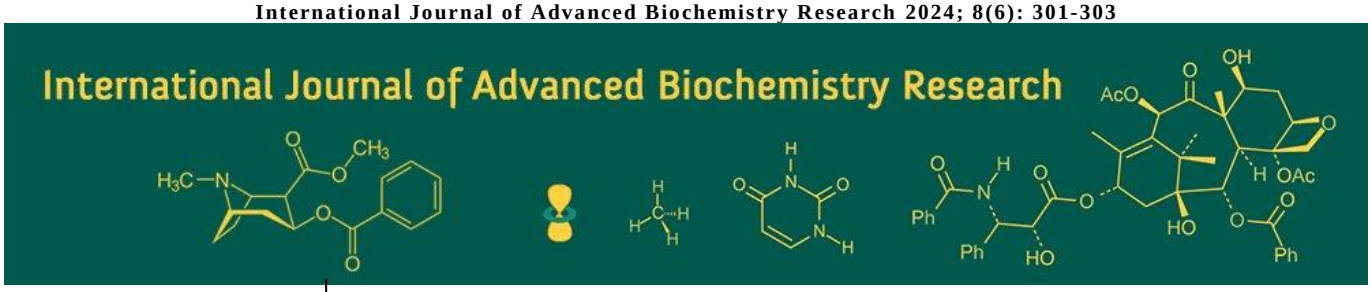

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Detection of extended spectrum beta lactamase *Escherichia coli* from chicken meat in Hisar district

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Abstract
This study focused on evaluating the occurrence of extended spectrum beta-lactamase (ESBL) producing *E. coli* in foods of animal origin *viz*., raw chicken meat. A total of 43 *E. coli* isolates from 63 isolates were positive for ESBL producing *E. coli.* Present findings highlight the importance of dissemination of ESBL type of antibiotic resistance into the foods of animal origin. To minimize contamination by foodborne pathogens, innovative, effective, and practical food safety controls and surveillance methods for multi-drug resistant foodborne pathogens are essential.

Keywords: Extended spectrum beta lactamase, *Escherichia coli*, chicken meat

Introduction
Animal-based foods, including milk and meat, are staple components of the human diet due to their high nutritional value. However, this same quality also makes them susceptible to rapid bacterial growth, particularly in settings with poor hygiene, such as in developing countries like India. The presence of pathogenic bacteria in these foods poses significant public health risks. Serious outbreaks of foodborne illnesses are often linked to the consumption of contaminated animal products, with common pathogens including *Escherichia coli*, Salmonella, Listeria monocytogenes, and Campylobacter spp (Quigley *et al*., 2013; Cerva *et al*., 2014) [14, 2]. Among foodborne pathogens, *Escherichia coli* is an opportunistic bacterium that infects both humans and animals, causing a variety of illnesses such as diarrhea, hemolytic uremic syndrome, and hemorrhagic colitis (Lanjewar *et al*., 2010) [10]. Treating *E. coli* infections has become more challenging due to the growing resistance to most first-line antimicrobial agents. Over time, resistance to cephalosporins in Enterobacteriaceae has risen, largely because of the spread of extended spectrum beta-lactamases (ESBLs) (Rasheed *et al*., 2014) [16]. ESBLs are a quickly evolving group of plasmid-mediated beta-lactamase enzymes capable of hydrolyzing and conferring resistance to penicillins, as well as first, second, and third-generation cephalosporins (such as cefotaxime and ceftazidime), and monobactams (Jhandai *et al*., 2022) [8]. In recent years, the widespread distribution of Enterobacteriaceae that produce extended spectrum beta-lactamases (ESBLs), particularly *E. coli*, has become a global issue in both healthcare and community environments. Raw meats from food animals carrying ESBL-producing bacteria may serve as a significant transmission route for these pathogens within communities (Hayashi *et al*., 2018) [6].

Materials and Methods
A total of 63 *E. coli* isolates obtained from 50 chicken meats samples were processed for detection of ESBLs (Jhandai *et al*., 2022) [8]. *E. coli* was ESBL screening was conducted using sensitivity tests with cefpodoxime, ceftazidime, aztreonam, cefotaxime, and ceftriaxone. A fresh pure culture was used to prepare a suspension of the test organism matching the 0.5 McFarland Standard. This suspension was spread over the entire surface of a Mueller Hinton agar plate using a sterile cotton swab. Various antimicrobial discs were then placed on the inoculated plate, maintaining adequate distance between them, and the plate was incubated at 37 °C for 18-24 hours. *Klebsiella pneumoniae* (ATCC 700603) from Hi-Media served as the beta-lactamase positive reference strain.

In the combination disc test, discs with cephalosporin alone (cefotaxime, ceftazidime) and in combination with clavulanic acid were used. The inhibition zone around the cephalosporin disc with clavulanic acid was compared to the zone around the disc with cephalosporin alone. A test is considered positive if the inhibition zone diameter is at least 5 mm larger with clavulanic acid than without it (CLSI, 2012).

Table 1: Criteria for ESBL confirmation

Method	Antibiotic Disc		Confirmation is positive if
Combination Disc Test (CDT)	Cefotaxime	CTX-30 mcg	≥ 5 mm increase in inhibition zone of cephalosporin with Clavulanic acid
	Cefotaxime with Clavulanic acid	CEC-30/10 mcg	
	Ceftazidime	CAZ-30 mcg	
	Ceftazidime with Clavulanic acid	CAC-30/10 mcg	

Results and Discussion

Of the 63 *E. coli* isolates, 43 (68.25%) samples were observed as ESBL producers based on the combination disc method.

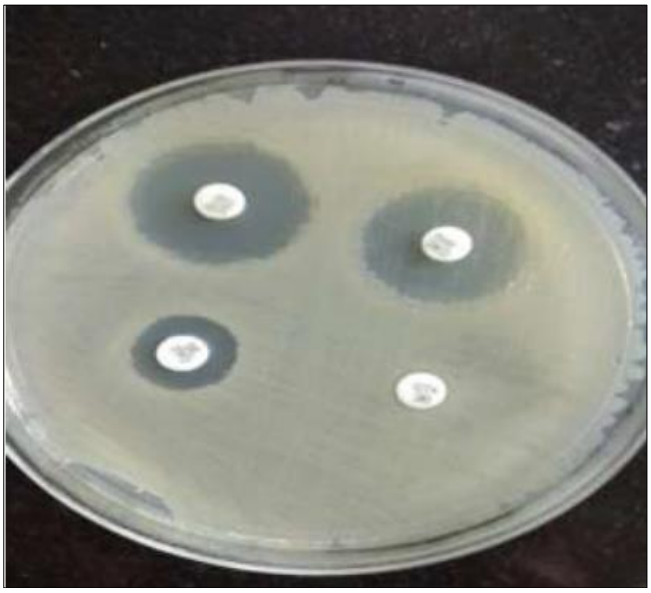

Fig 1: Combined disc test for confirmation of extended spectrum beta-lactamase *E. coli*

In present study, 43 (68.25%) isolates out of 63 of meat origin were phenotypically positive for ESBL. The high ESBL occurrence determined for all investigated foods of animal origin in this study is surprising. Nevertheless, one reason could be the use of beta-lactams and even 4th generation cephalosporins - in veterinary medicine (Geser *et al*., 2012) [5]. The high prevalence rate of ESBL producers recovered from food samples might well depend on frequent horizontal gene transfer between bacterial isolates, through exchanges of plasmids and/or mobile elements carrying ESBL genes (Kawamura *et al*., 2014) [9]. Raw meat available in local retail shops without appropriate temperature control and poor sanitary measures favor the contamination by *E. coli* and other organisms (Dewangan *et al*., 2016) [4]. The findings of present study are in line with various studies (except a few) like Onen *et al*., 2015 [12] in China (60.78%), Overdevest *et al*., 2011 [13] in Netherland (76.8%), Le *et al*., 2015 [11] in Vietnam (58.7%) and Randall *et al*., 2016 in UK (62.30%), whereas Bhoomika *et al*., 2016 [1] in Chhattisgarh, India, Hayashi *et al*., 2018 [6] in Japan and Hussain *et al*., 2017 from various part of India have reported comparatively lower prevalence of ESBL ranging from 3.07% to 40.3%, Silva *et al*., 2012 [17] in Portugal, Onen *et al*., 2015 [12] in Turkey have reported much higher prevalence of up to 93% *E. coli* in chicken meat.

Conclusion

This study showed that high prevalence of ESBL producing *E. coli* in foods of animal origin emphasize the importance of livestock and poultry as a reservoir for such organisms. We found that out of 63 *E. coli* isolates, 43 (68.25%) were observed to be ESBL producers, such high prevalence is threat to both human and animals.

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