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Escherichia coli prevalence and antibiograms identified in meat or meat products

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Abstract
An essential pathogen that is found in food is *Escherichia coli*. It serves as a facultative anaerobic bacterium that is harmful to both humans and animals and is part of normal microbial flora of digestive tract. Diarrheagenic *E. coli* are those that cause diarrhea along with other enteric illnesses. Although many strains of *E. coli* colonize the intestine innocuously, others cause a variety of intestinal illnesses. *E. coli* contamination of meat as well as meat products poses a risk to public health. 100 samples of RTE meat products (30), chevon (20), mutton (20), and chicken meat (30) were gathered from the Rajasthani city of Udaipur. The chicken, mutton, chevon, and RTE meat products all had *E. coli* incidence levels of 56.66% (17), 45% (9), 40% (8), and 13.33% (4), respectively. Antibiotic susceptibility testing against ten different antibiotics were also performed on all *E. coli* isolates of meat/meat products. The results of the antibiogram study showed that the isolates were susceptible to ceftriaxone (78.95%), gentamicin (78.95%), and ampicillin (63.16%). While erythromycin (94.74%), Amoxicillin+Clavulanic acid (68.4%), and Oxytetracycline (68.4%) were all shown to be resistant to the isolates.

Keywords: Meat products, *E. coli*, prevalence, antibiogram

Introduction
Worldwide public health challenges include microbial food safety and food-borne illnesses [9]. The high biological value, low cost, wonderful flavor, and ease of preparation, meat and or ready-to-eat (RTE) meat products are in highly demanded [32, 39]. They are also excellent source of good quality proteins, mineral, and vitamin. The World Health Organization claims that a significant part of diarrhea around the world is brought on by eating food that has been tainted. The Center for Disease Control and Prevention (CDC), USA, estimates that the *Escherichia coli* is a contributing factor in 76 million instances of foodborne disease [13]. The family Enterobacteriaceae includes *E. coli*. It is a short, Gram-negative, facultatively anaerobic, motile bacterium. One of the typical intestinal bacterial flora seen in poultry that is harmful to both people and animals is *E. coli* [7, 28]. Diarrheagenic *E. coli* are those that cause diarrhea and other enteric illnesses. Although many *E. coli* strains cause different intestinal illnesses, they colonize the intestines without causing any harm [40]. A kidney infection, septicemia, pneumonia, meningitis, hemorrhagic colitis, severe food poisoning, hemolytic uremic syndrome, abdominal pains, vomiting, dysentery, and bloody or non-bloody diarrhea are all possible effects of *E. coli* [22]. *E. coli* are naturally found in ruminants, such as sheep and goats. The infection also present on the animal's skin or in its feces at the time of slaughter, and it may transfer to the carcass by evisceration or skin removal process. Therefore, using subpar and unclean slaughter methods significantly raises the risk of *E. coli* contamination in meat [27]. The prevalence of multiple drug resistance of *E. coli* has increased due to the careless use of antibiotics. The use of antibiotics in the production of food animals has significant effects on human Health's. Additionally, *E. coli* is regarded as an indicator bacterium for Enterobacteriaceae family members' antibiotic resistance [30]. Many antibiotics may not be effective against *E. coli*. But *E. coli* isolates from poultry are typically antibiotic-resistant, especially they have widely utilized in the poultry business for a long time [12]. *E. coli* that is now a serious risk to both human and animal health is antibiotics-resistant to two and or more classes [21].

Materials and Methods

A total, 100 samples of meat or meat products were taken from the city of Udaipur. Two times every week, meat outlets and stores in the Rajasthani city of Udaipur were visited to gather samples of meat and RTE meat products. The samples were collected in a sterile container and brought to the lab in a refrigerated state using ice packs within two hours.

E. coli isolation and identification

E. coli were isolated from meat and or meat products using the procedure outlined by [29, 34]. For enrichment, the 25grams of sample was added to 225 ml of MacConkey broth after being properly triturated in a sterile mortar and pestle. A loopful of inoculum was plated on MacConkey agar and incubated at 37 °C for 24 hours after the culture flask had been cultured at 37 °C for 24 hours. The pink (lactose-fermenter) colonies were selected after 24 hours and streaked over eosin methylene-blue agar (EMB). Colonies with a metallic green gloss were chosen for additional verification.

Morphological characteristics

Gram negative bacilli that were grouped singly or in pairs were seen when the Gram-stained smear was examined under a microscope.

Biochemical evaluation

Numerous biochemical assays, including the indole test, methyl-red test, Voges-proskauer-test, citrate-utilization test, TSI test, sugar-fermentation test, catalase-test, oxidase-test, and urease-test, were carried out to confirm the suspected *E. coli* isolates [24, 31].

Antibiotic-susceptibility test

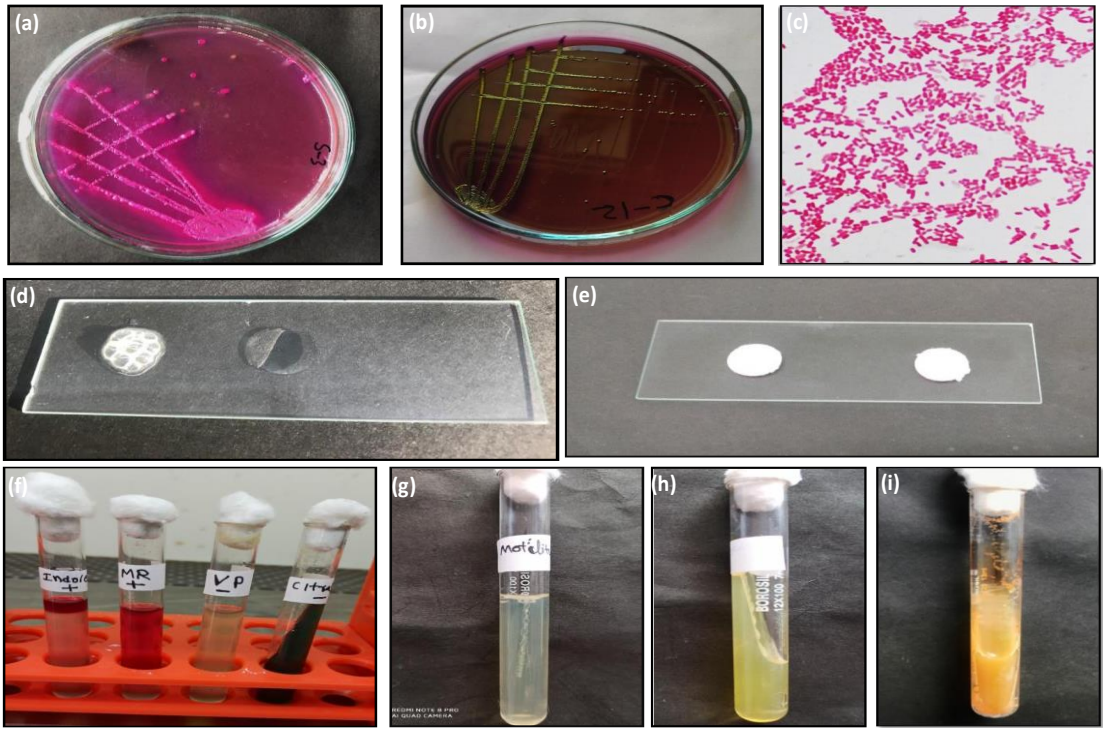
Antibiotic-susceptibility tests was performed on each and every *E. coli* isolate in accordance with [8]. The agar-disc diffusion method was used to test the susceptibility to an antibiotics. The test culture was isolated, and one isolated colony was injected in Luria-Bertani-broth and incubated for 24 hours at 37 °C. After that, a Mueller-Hinton agar plate was covered with the swab culture. The inoculated agar plate was covered with the antibiotic discs. To make sure that every disc made complete contact with the agar surface, each was manually pressed down. A total of 10 antibiotic discs: amoxyclav; ampicillin; ceftriaxone; chloramphenicol; cotrimoxazole; enrofloxacin; erythromycin; gentamicin; nalidixic acid; and oxytetracycline. Following incubation, the diameter of the zone of inhibition was evaluated to ascertain the isolates' patterns of antibiotic susceptibility. According to the procedure outlined by [16, 26], the prevalence of multi-drug resistant *E. coli* was assessed.

Results

The test isolate that formed pink-colonies on MacConkey agar were chosen and streaked on EMB agar later. The 38 isolates with a green metallic sheen were identified from 100 samples and identified using Gram's staining and biochemical assays. The isolate was morphologically recognized as Gram negative- bacilli when the Gram's staining was done, and they were organized single or in pairs. Each of the 38 isolates underwent several biochemical assays after first isolation. In indole and methyl-red test, all suspicious isolates (n=38) developed a red ring on top and a red color, respectively. Similar to this, no growth of red or blue color was seen during the Voges-proskauer test or citrate test, respectively. This revealed that while citrate and the Voges-Proskauer test were negative for all of the suspicious isolates, they were all positive for the indole and MR tests. Fig. 1a-1i provide descriptions of the outcomes of several biochemical tests. All 100 samples of meat and RTE meat products, including chicken meat, mutton, chevon, and RTE meat products, were examined. Of these, 17, 9, 8, and 4 samples were found to be positive for *E. coli*, giving the prevalence rates of 56.65%, 45.00%, 40.00%, and 13.34%, respectively. *E. coli* was determined to be prevalent overall at 38.00% (38/100). The most effective antibiotics against the 38 isolates found in the various meat samples were chloramphenicol (84.22%), gentamicin (78.95%), ceftriaxone (78.95%), and ampicillin (63.16%). Erythromycin resistance was discovered in the isolates (94.73%). Additionally, moderately high resistance to oxytetracycline (36.45%) and amoxicillin/clavulanic acid (36.45%) was found in the current investigation. Table No. 2 displays the results of the antibiotic-susceptibility pattern of the *E. coli* isolates isolated from meat and RTE meat products.

Table 1: Antibiotic-susceptibility of *E. coli* isolates

Name of antibiotics	Chicken (17 isolates)			Mutton (9 isolates)			Chevon (8 isolates)			RTE products (4 isolates)		
	S	I	R	S	I	R	S	I	R	S	I	R
Gentamicin	13 (76.4%)	4 (23.5%)	0 (0%)	7 (77.8%)	1 (11.1%)	1 (11.1%)	6 (75%)	2 (25%)	0 (0%)	4 (100%)	0 (0%)	0 (0%)
Ceftriaxone	13 (76.4%)	2 (11.7%)	2 (11.7%)	7 (77.7%)	1 (11.1%)	1 (11.1%)	7 (87.5%)	0 (0%)	1 (12.5%)	3 (75%)	1 (25%)	0 (0%)
Nalidixicacid	44 (23.5%)	2 (11.7%)	11 (64.7%)	7 (77.7%)	1 (11.1%)	1 (11.1%)	6 (75%)	1 (12.5%)	1 (12.5%)	2 (50%)	2 (50%)	0 (0%)
Enrofloxacin	5 (29.4%)	6 (35.2%)	6 (35.2%)	8 (88.8%)	0 (0%)	1 (11.1%)	6 (75%)	1 (12.5%)	1 (12.5%)	4 (100%)	0 (0%)	0 (0%)
Ampicillin	8 (47.0%)	0 (0%)	9 (52.9%)	7 (77.7%)	0 (0%)	2 (22.2%)	6 (75%)	1 (12.5%)	1 (12.5%)	3 (75%)	1 (25%)	0 (0%)
Chloram-phenicol	11 (64.7%)	1 (5.8%)	5 (29.4%)	9 (100%)	0 (0%)	0 (0%)	8 (100%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)	0 (0%)
Erythro-mycin	0 (0%)	0 (0%)	17 (100%)	0 (0%)	1 (11.1%)	8 (88.8%)	0 (0%)	1 (12.5%)	7 (87.5%)	0 (0%)	0 (0%)	4 (100%)
Amoxicillin/clavulanic Acid	5 (29.4%)	3 (17.6%)	9 (52.9%)	3 (33.3%)	3 (33.3%)	3 (33.3%)	6 (75%)	0 (0%)	2 (25%)	4 (100%)	0 (0%)	0 (0%)
Co-Trimoxazole	10 (58.8%)	2 (11.7%)	5 (29.4%)	8 (88.8%)	0 (0%)	1 (11.1%)	7 (87.5%)	0 (0%)	1 (12.5%)	3 (75%)	0 (0%)	1 (25%)
Oxy-tetracycline	6 (35.2%)	0 (0%)	11 (64.7%)	7 (77.7%)	0 (0%)	2 (22.2%)	7 (87.5%)	0 (0%)	1 (12.5%)	4 (100%)	0(0%)	0 (0%)



Identification of E.coli : (a) Growth of the test culture on MacConkey agar plate. **(b)** Growth of the test culture on eosin methylene blue agar (EMB). **(c)** Gram's staining of the isolates (Gram negative rods). **(d)** Oxidase test (-ve). **(e)** Catalase test (+ve). **(f)** IMViC test of the isolates (++--). **(g)** Motility test of the isolates **(h)** TSI test (positive). **(i)** Urease test (negative)of the isolates.

According to our investigation, 56.66% of chicken meat samples included *E. coli*, which is comparable to the prevalence reported by [5, 11, 36] who reported 57.01%, 53.57%, 66.32%, and 61.76%, respectively. [17, 24, 38, 42] reported a significantly lower prevalence of 41.40%, 31.00%, 37.00%, and 34.00%, respectively. While a greater prevalence-rate of 93.75%, 83.5%, and 87.5%, respectively, was reported by [3, 18, 43]. Our study's somewhat high prevalence rate of E. coli in meat, and/or RTE meat products is a sign of unhygienic conditions surrounding the killing and sale of the animals. Additionally, unsanitary handling and shipping of meat products can be blamed for infection in those goods. Additionally, untrained and disorganized butchers produce and market the majority of the meat in the study area. This highlights the requirement to educate the public and butchers on hygienic meat production procedures.

Chloramphenicol (84.2%), Gentamicin (78.9%), Ceftriaxone (78.9%), and Ampicillin (63.1%) were the antibiotics most efficient against the 38 isolates found in the various meat samples. According to [2, 17, 33, 35], similar susceptibility values against chloramphenicol were 81.54%, 85.1%, 82.67%, and 67.1%, respectively. Conversely, [4, 19, 41] found that 79.4%, 73%, and 58% of the samples they tested showed resistance to chloramphenicol. Similar to this, [17, 33] found that antibiotic-sensitivity to gentamicin was 85.7% and 81.0%, respectively. The resistance to gentamicin was found to be 46.6% and 57.47%, respectively, by [1, 20]. Ceftriaxone was shown to be effective to *E. coli* isolates in 73% and 94.29% of the cases, respectively, according to [23, 33]. The effectiveness of ampicillin against 52.9% of the isolates was also reported by [2]. Although [10, 16, 19, 25, 37] found that resistance to ampicillin was seen to be 57%, 84.93%, 98%, 80.43%, 63.4%, 62%, and 75.6%, respectively. The erythromycin resistance of the E. coli isolates isolated. However, there was also evidence of fairly high resistance to oxytetracycline and amoxicillin + clavulanic acid. The widespread and careless use of antibiotics to treat illnesses in humans and promote animal growth is highlighted by this rising resistance. A significant source of ESBL-producing E. coli for humans is poultry and chicken meat. These drug-resistant *E. coli* can directly or indirectly infect people through food, and they may also spread resistance genes to susceptible bacteria. In order to reduce the risk to the public health posed by the growth of antibiotic-resistant bacteria, careful use of antibiotics is important.

Conclusion

In this investigation, 100 samples of meat and RTE meat products were gathered from various retail meat stores in Udaipur, and the prevalence in *E. coli* was reported to be 38% (38/100). Total *E. coli* isolates found in chicken meat, mutton, chevon, and RTE meat products were 17, 9, 8, and 4, respectively, with prevalence rates of 56.66%, 45%, 40%, and 13.33%. Out The most effective antibiotics for the 38 isolates recovered from the various meat samples were ampicillin (63.15%), gentamicin (78.94%), chloramphenicol (84.21%), and ceftriaxone (78.94%). Erythromycin resistance was discovered in the isolates (94.73%). Additionally, in the current investigation, moderately high resistance to oxytetracycline (36.84%) and amoxicillin/clavulanic acid (36.84%) was observed. These findings highlight the necessity of using antibiotics sparingly in animal husbandry and the threat to the general public's health posed by the proliferation of bacteria that are resistant to them and cause food-borne illnesses.

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