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Prevalence and antibiogram of *Escherichia coli* isolated from meat and meat products

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Abstract
Escherichia coli is an important food-borne pathogen. It is a facultative anaerobic bacterium among the common microbial flora of gastrointestinal tract which is pathogenic to humans and animals. *E. coli* that cause diarrhea and other enteric illness are called diarrheagenic *E. coli*. It harmlessly colonizes the intestine but many strains of *E. coli* cause various intestinal diseases. Presence of *E. coli* in meat and meat products is a public health hazard. A total of 100 samples comprising of chicken meat (30), mutton (20), chevon (20) and RTE meat products (30) were collected from the Udaipur city in Rajasthan. The prevalence of *E. coli* were recorded in chicken meat, mutton, chevon and RTE meat products as 56.66% (17), 45% (9), 40% (8) and 13.33% (4) respectively. All the isolates obtained from meat and RTE meat products were also subjected to antibiotic susceptibility test against 10 different antibiotics. The analysis of antibiogram revealed that the isolates were sensitive for chloramphenicol (84.21%), gentamicin (78.94%), ceftriaxone (78.94%) and ampicillin (63.15%) While, the isolates were found to be resistant to erythromycin (94.73%), amoxicillin/clavulanic acid (36.84%) and oxytetracycline (36.84%).

Keywords: Meat, meat products, *Escherichia coli,* prevalence, antibiogram

Introduction
Microbial food safety and food-borne infections are global public health concern. In the 21th century, food borne diseases have become one of pertinent issue all over the world [9].Meat and Ready to eat (RTE) meat products are in high demand due to their high biological value, low price, good taste and easily serving, as well as they are excellent sources of high quality protein, minerals and vitamins [32, 39]. According to the World Health Organization, a major proportion of diarrhoea in the world occurs due to the consumption of contaminated food. According to the Center for Disease Control and Prevention (CDC), USA, there are 76 million cases of food borne illness associated with various food borne organisms including *Escherichia coli* [13]. *E. coli* is a member of Enterobacteriaceae family. It is a short, Gram negative, non-spore forming and motile bacterium with peritrichous flagella. It is a facultative anaerobic bacterium. *E. coli* is one of the common microbial flora of gastrointestinal tract of poultry which is pathogenic to humans and animals [28, 7]. *E. coli* that cause diarrhea and other enteric illness are called diarrheagenic *E. coli*. It harmlessly colonizes the intestine but many strains of *E. coli* cause various intestinal diseases [40]. *E. coli* can cause haemorrhagic colitis, severe food poisoning, hemolytic uremic syndrome, bloody diarrhea, non-bloody diarrhea, abdominal cramps, vomiting, dysentery, kidney infection, septicemia, pneumonia and meningitis [22]. The ruminants, including sheep and goats are natural reservoirs for *E. coli*. During slaughter, the pathogen may be present on the skin or in the feces of the animal and may get transferred to the carcass during evisceration or skin removal. Therefore, poor and unhygienic slaughter techniques greatly increase the risk of meat contamination with *E. coli* [27].

The non-judicious use of antibiotics and antimicrobials has increased the incidence of multiple drug resistance in *E. coli*. The use of antibiotics in the food-animal production has major implications for human health. It is also considered as an indicator organism for antimicrobial resistance in the members of Enterobacteriaceae family [30]. *E. coli* may be sensitive to many antibiotics. However, isolates of *E. coli* from poultry are frequently resistant to one or more antibiotics, especially if they have been widely used in poultry industry over a long period [12] *E. coli* which is resistant to two or more classes of antibiotics is now an important human and animal health hazard [21].

Materials and Methods

Sample collection

A total of 100 samples of meat and meat products were collected from Udaipur city. The samples of meat and ready to eat meat products were collected twice in a week from meat outlets and shops from Udaipur city in Rajasthan. The samples were collected in sterile container and transported to the laboratory within 2 hours in chilled condition by using ice packs.

Isolation and identification of *Escherichia coli*

Isolation of *E. coli* from the samples of meat and meat products was done as per the method described by [34, 29]. Twenty five grams of meat and meat product sample was weighed aseptically. After proper trituration in sterile mortar and pestle, the sample was added to 225 ml of MacConkey broth for enrichment. The culture flask was incubated at 37 ^{0}C for 24 hrs, followed by plating of a loopful of inoculum on MacConkey agar and incubated at 37 ^{0}C for 24 hours. After 24 hours, pink coloured (lactose fermenter) colonies were picked up and streaked on eosin methylene blue agar (EMB). The colonies showing green metallic sheen were selected for further confirmation.

Morphological characteristics

The Gram stained smear was observed under microscope which revealed Gram negative bacilli which were arranged singly or in pairs.

Biochemical examination

Various biochemical tests were performed to confirm the suspected *E. coli* isolates viz., indole production test, methyl red test, voges-proskauer test, citrate utilization test, TSI test, sugar fermentation test, catalase test, oxidase test and urease test [24, 31].

Antibiotic Susceptibility test

All the *E. coli* isolates were subjected to antibiotic susceptibility test as described by [8]. Antibiotic susceptibility testing was done by agar disc diffusion method. A single isolated colony of the test culture was inoculated in Luria Bertani broth & incubated at 37 ºC for 24 hrs. Then, the swab culture was smeared on Mueller Hinton agar plate. The antibiotic discs were placed on the surface of the inoculated agar plate. Each disc was pressed down individually to ensure complete contact with the agar surface. A total of 10 antibiotic discs comprising of amoxyclav, ampicillin, ceftriaxone, chloramphenicol, co-trimoxazole, enrofloxacin, erythromycin, gentamicin, nalidixic acid and oxytetracycline were placed on two agar plates each containing 5 antibiotic discs. After incubation, the diameter of the zone of inhibition was measured so as to determine the antibiotic susceptibility pattern of the isolates for the respective antibiotics. The prevalence of MDR (multi drug resistant) *E. coli* was evaluated according to the method described by [16, 26].

Results

The test isolates which produced pink coloured colonies on MacConkey agar were selected and were further streaked on EMB agar. Out of 100 samples, 38 isolates showing green metallic sheen were picked up and characterized by Gram's staining and biochemical tests. On performing the Gram's staining, the isolates were morphologically identified as Gram negative bacilli arranged singly or in pairs. After preliminary isolation, all the 38 isolates were subjected to different biochemical tests. All the suspected isolates (n=38) when tested for indole & methyl red (MR) test, showed the development of red coloured ring on the top and red colour, respectively. Similarly, on testing for voges- proskauer test and citrate test**,** no development of red and blue colour was observed, respectively. This indicated that all the suspected isolates were positive for indole and MR test, while negative for citrate and voges-proskauer test. The results of various biochemical tests are described in Table No.1 and Fig No. 1a-1i.

Table 1: Biochemical reactions of the isolates

S. No.	Biochemical Test	Reactions
1.	Indole test	+ve
2.	Methyl red test	+ve
3.	Voges-Proskauer test	-ve
4.	Citrate test	-ve
5.	Sugar fermentation test (glucose)	+ve
6.	Urease test	-ve
7.	TSI test	yellow colour of slant and butt
8.	Catalase test	+ ve
9.	Oxidase test	-ve
10.	Motility test	Motile

On the examination of all the 100 meat and RTE meat products samples collected from different retail meat shops in Udaipur such as chicken meat (n=30), mutton (n=20), chevon (n=20) and RTE meat products (n=30), 17, 9, 8 and 4 samples were found to be positive for *E. coli* giving a prevalence rate of 56.66%, 45%, 40% and 13.33%, respectively. In total, the prevalence of *E. coli* was found to be 38% (38/100).

Out of the 38 isolates recovered from the different meat samples, the most effective antibiotics were chloramphenicol (84.21%), gentamicin (78.94%), ceftriaxone (78.94%) and ampicillin (63.15%). While, the isolates were found to be resistant to erythromycin (94.73%). Also, in the present study, moderately high resistance was shown towards amoxicillin/clavulanic acid (36.84%) and oxytetracycline (36.84%). The results of the antibiotic susceptibility pattern of the *E. coli* isolates recovered from meat and RTE meat products are shown in Table No 2 and Fig No 2.

Table 2: Antibiotic susceptibility pattern of *E. coli* isolates recovered from chicken, mutton, chevon and RTE meat product samples

Name of antibiotics	Chicken (17 *E. coli*)			Mutton (9 *E. coli*)			Chevon (8 *E. coli*)			RTE products (4 *E. coli*)		
	S	I	R	S	I	R	S	I	R	S	I	R
Gentamicin	13(76.47%)	4(23.52%)	0(0%)	7(77.77%)	1(11.11%)	1(11.11%)	6(75%)	2(25%)	0(0%)	4(100%)	0(0%)	0(0%)
Ceftriaxone	13(76.47%)	2(11.76%)	2(11.76%)	7(77.77%)	1(11.11%)	1(11.11%)	7(87.5%)	0(0%)	1(12.5%)	3(75%)	1(25%)	0(0%)
Nalidixic acid	44(23.52%)	2(11.76%)	11(64.70%)	7(77.77%)	1(11.11%)	1(11.11%)	6(75%)	1(12.5%)	1(12.5%)	2(50%)	2(50%)	0(0%)
Enrofloxacin	5(29.41%)	6(35.29%)	6(35.29%)	8(88.88%)	0(0%)	1(11.11%)	6(75%)	1(12.5%)	1(12.5%)	4(100%)	0(0%)	0(0%)
Ampicillin	8(47.05%)	0(0%)	9(52.94%)	7(77.77%)	0(0%)	2(22.22%)	6(75%)	1(12.5%)	1(12.5%)	3(75%)	1(25%)	0(0%)
Chloramphenicol	11(64.70%)	1(5.88%)	5(29.41%)	9(100%)	0(0%)	0(0%)	8(100%)	0(0%)	0(0%)	4(100%)	0(0%)	0(0%)
Erythromycin	0(0%)	0(0%)	17(100%)	0(0%)	1(11.11%)	8(88.88%)	0(0%)	1(12.5%)	7(87.5%)	0(0%)	0(0%)	4(100%)
Amoxicillin/clavulanic acid	5(29.41%)	3(17.64%)	9(52.94%)	3(33.33%)	3(33.33%)	3(33.33%)	6(75%)	0(0%)	2(25%)	4(100%)	0(0%)	0(0%)
Co-Trimoxazole	10(58.82%)	2(11.76%)	5(29.41%)	8(88.88%)	0(0%)	1(11.11%)	7(87.5%)	0(0%)	1(12.5%)	3(75%)	0(0%)	1(25%)
Oxytetracycline	6(35.29%)	0(0%)	11(64.70%)	7(77.77%)	0(0%)	2(22.22%)	7(87.5%)	0(0%)	1(12.5%)	4(100%)	0(0%)	0(0%)

S= sensitive, I= intermediate, R= resistant

Fig 1a: Growth of the test culture on MacConkey agar plate

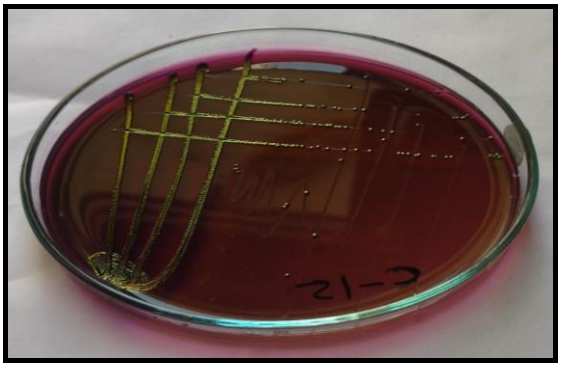

Fig 1b: Growth of the test culture on eosin methylene blue agar (EMB)

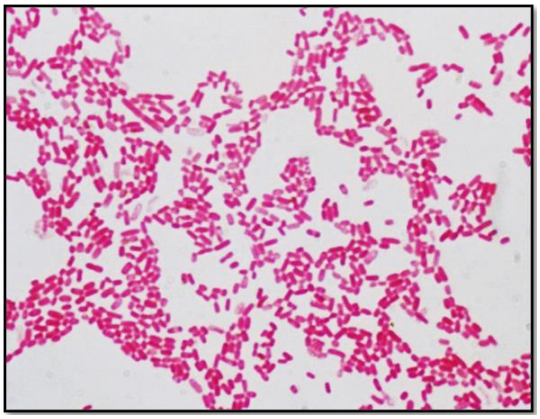

Fig 1c: Gram's staining of the isolates (Gram negative rods)

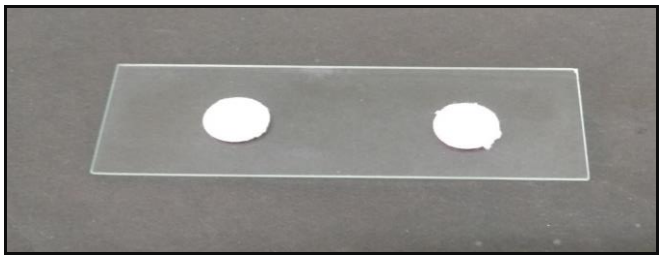

Fig 1d: Oxidase test (-ve)

Fig 1e: Catalase test (+ve)

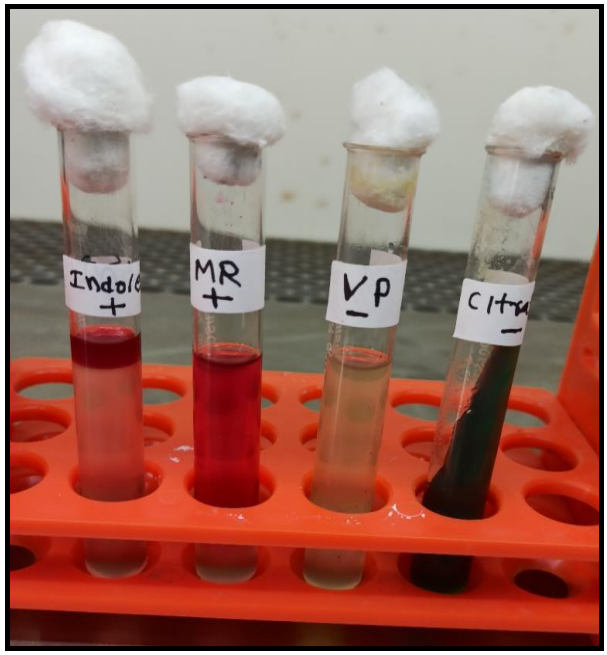

Fig 1f: IMViC test of the isolates (++--)

Fig 1g: Motility test of the isolates

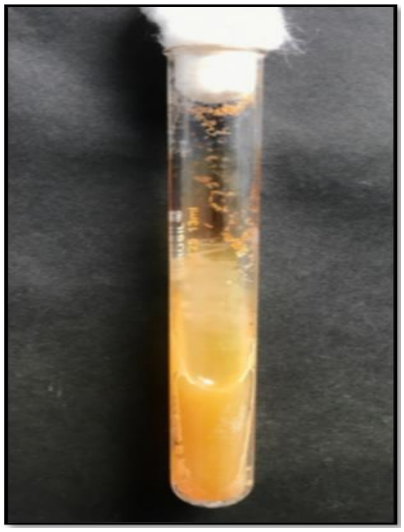

Fig 1h: TSI test (positive) of the isolates

Fig 1i: Urease test (negative) of the isolates

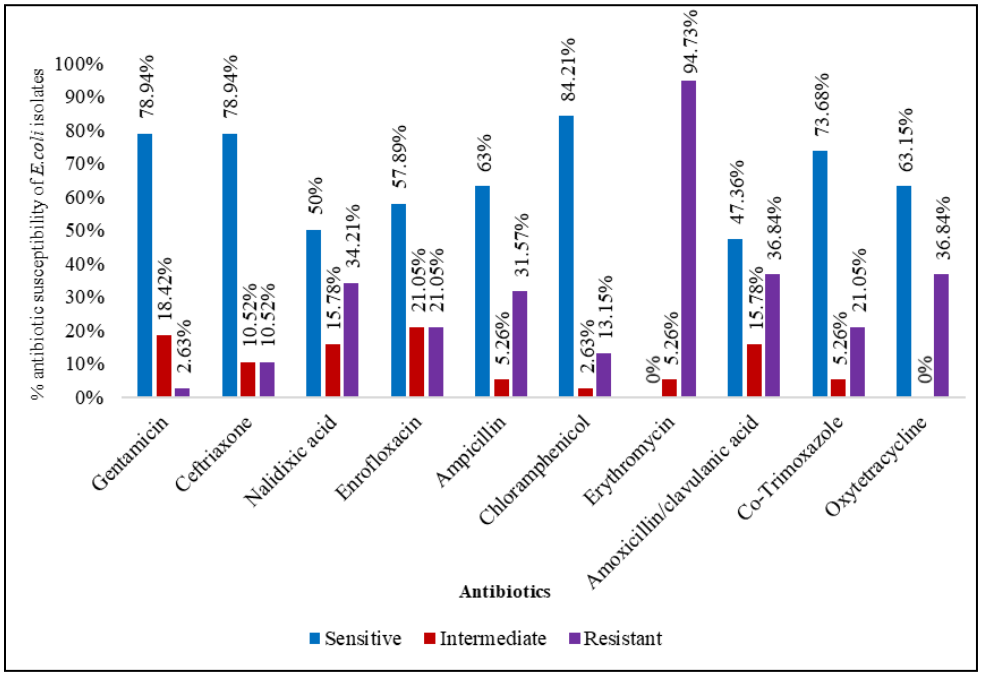

Fig 2: Antibiogram of *E. coli* isolates recovered from different meat sources

Discussion

In our study, the prevalence of *E. coli* in chicken meat samples was found to be 56.66% which was similar to the prevalence reported by [14, 5, 11, 36] who reported the prevalence

as 57.01%, 53.57%, 66.32% and 61.76%, respectively. A significantly lower prevalence was reported by [42, 38, 17, 24] as 41.4%, 31%, 37% and 34%, respectively. Whereas, higher prevalence rate were reported by [3, 43, 18] as 93.75%, 83.5% and 87.5%, respectively.

Moderately high prevalence rate of *E. coli* in meat and RTE meat products in our study indicates towards poor sanitary environmental conditions under which the animals are slaughtered and sold. Also, the contamination in meat products can be attributed to unhygienic handling and transportation of these products. Moreover, in the study area the majority of meat is produced and marketed by unskilled and unorganized butchers. This emphasizes the need to create awareness among the butchers as well as the public regarding the hygienic meat production practices.

Out of the 38 isolates recovered from the different meat samples, the most effective antibiotics were chloramphenicol (84.21%), gentamicin (78.94%), ceftriaxone (78.94%) and ampicillin (63.15%). Similar susceptibility results were reported by [35, 2, 33, 17] as 81.54%, 85.1%, 82.67% and 67.1% respectively against chloramphenicol. On the other hand, contrasting findings revealing resistance against chloramphenicol were observed by [19, 4, 41] as 79.4%, 73% and 58%, respectively. Similarly, [33, 17] reported antibiotic sensitivity against gentamicin as 85.72% and 81% respectively. While, [1, 20] reported 46.6% and 57.47%, resistance pattern against gentamicin, respectively. Also, [23, 33] observed ceftriaxone as effective against 73% and 94.29% *E. coli* isolates, respectively. Further, [2] reported ampicillin as effective against 52.9% of the isolates. While, [16, 10, 37, 25, 19] observed resistance against ampicillin as 57%, 84.93%, 98%, 80.43%, 63.4%, 62% and 75.6%, respectively.

The *E. coli* isolates recovered from meat and RTE meat products were found to be highly resistant to erythromycin. While, moderately high resistance towards amoxicillin/clavulanic acid and oxytetracycline was also evident. This increasing resistance highlights the widespread and non-judicious use of antibiotics for the treatment of human diseases as well as for growth promotion in animals. Poultry and chicken meat are an important source of ESBL producing *E. coli* for humans. This put emphasis on the need to address the risk associated with ESBL production. These antibiotic resistant *E. coli* can infect humans directly or indirectly with food and may also transfer resistance gene to susceptible bacteria. Thus, prudent use of antibiotics is the need of the hour to prevent the public health risk arising due to the emergence of antibiotic resistant pathogens.

Conclusion

In this study, the prevalence of *E. coli* was found to be 38% (38/100) among the 100 meat and RTE meat products collected from different retail meat shops in Udaipur. In total, 17, 9, 8 and 4 isolates of *E. coli* were recovered from chicken meat (n=30), mutton (n=20), chevon (n=20) and RTE meat products (n=30), giving a prevalence rate of 56.66%, 45%, 40% and 13.33%, respectively. Out of the 38 isolates recovered from the different meat samples, the most effective antibiotics were chloramphenicol (84.21%), gentamicin (78.94%), ceftriaxone (78.94%) and ampicillin (63.15%). While, the isolates were found to be resistant to erythromycin (94.73%). Also, in the present study, moderately high resistance was shown towards amoxicillin/clavulanic acid (36.84%) and oxytetracycline (36.84%). These results signify the importance of judicious use of antibiotics in animal husbandry and the public health risk arising from the spread of antibiotic resistant bacteria leading to food borne illness.

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