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Statistical analysis among different meat samples processed for *E. coli*

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Abstract
The present research work was conducted on 270 meat samples for isolation of *E. coli*. During the study a total of 270 meat samples were processed which included 210 meat samples and 60 swab samples. Results revealed 63 samples (23.33%) which comprised of 51/210(24.28%) from meat and 12/60 (20%) swabs were positive for *E. coli.* Statistical analysis demonstrated relative risk of getting infection among the meat samples is in descending order of Carabeef, followed by Chicken and Chevon. The swab samples from butcher's knives and chopping board swabs have higher risk of propagating infection. Among both the meat and swab samples the risk of getting the infection is higher from the meat samples. The statistical results also revealed there was no association between the presence and absence *E. coli* and the type of meat, swab and overall occurrence.

Keywords: *E. coli*, meat, risk, odd's ratio

Introduction
Food borne disease conditions are widespread in developed as well as developing countries, as a growing public health problem and an economic burden. Gastroenteritis is the dominant indicator of food borne illnesses. Meat and meat products have been incriminated as a predominant cause of many food borne illnesses. With the change in the food habit of man and pace in the food processing industry, many food borne zoonotic pathogens have emerged, and *E. coli* is one of them. (Thanigaivel and Anandhan, 2015) [10].
Escherichia coli is the widely prevalent facultative anaerobic, Gram-negative, straight rods, measuring 1.1 - 1.5 mm diameter and 2.0 - 6.0 mm length, motile by peritrichous flagella and non-spore forming, mesophilic, lactose fermenting bacteria and a vital component of the intestinal flora of the healthy host. It belongs to the Enterobacteriaceae family, which also includes many other genera like *Shigella*, *Yersinia*, and *Salmonella*. They convert nitrate to nitrite, are oxidase negative and catalase positive. Most *E. coli* possess beta-glucoronidase, which digests complex carbohydrates. Non-pathogenic *E. coli* may act as opportunistic pathogen, infective to the immunocompromised hosts. The pathogenic strains of *E. coli* induce gastrointestinal illness (Feng, *et al*., 2002) [3] (Adamu *et al.*, 2014) [1].
More than 700 different serotypes of *E. coli* have already been identified using somatic (O), capsular (K), and flagellar (H) antigens and species are also bio typed as sorbitol-non-fermenting *E. coli* that causes food poisoning in human being, based on the sorbitol fermentation. Wide range of clinical manifestations caused by this biotype include moderate sickness, vomiting, diarrhea, hemolytic uremic syndrome, and mortality (Su and Brandt, 1995) [8].
Escherichia coli is a typical resident of the large intestine in humans and warm-blooded animals. Therefore, fecal contamination during the milking process together with poor hygiene practices might spread *E. coli* to raw milk and milk products. It primarily spreads to people by eating contaminated foods including raw or undercooked meat, unpasteurized milk and contaminated vegetables (Lara *et al*., 2016) [6].

Materials and Methods
The study was carried out in the department of Veterinary Public Health and Epidemiology, College of Veterinary science & AH, Kamdhenu University, Navsari from December 2022 to May 2023.

Meat samples

Using random sampling method, total 210 raw meat samples, each weighing 100 g, were collected aseptically from meat shops located in and around Navsari city; and the carabeef samples were procured from Deonar abattoir, BMC, Mumbai. The samples were collected in 3 x 4 cm sterile polyethylene bags with proper labeling, as mentioned in Table 1.

Table 1: Details of the meat samples

Sr. No.	Type of meat	Nature of the sample					Total
		Minced	Breast/Rib muscle	Thigh muscle	Wing muscle	Giblet/Pluck	
1.	Chicken	25	15	10	5	5	60
2.	Chevon	20	10	10	-	10	50
3.	Mutton	20	10	10	-	10	50
4.	Carabeef	25	15	5	-	5	50
Total		90	50	35	5	30	210

Swab samples

A total of 60 swab samples comprising of 20 samples each, from butcher's hand, butcher's knife and chopping board were collected using commercial sterile cotton swab sticks, as mention in Table 2.

Table 2: Details of the Swab samples

Sr. No.	Type Source	Chicken shop	Chevon shop	Mutton shop	Total
1.	Butcher's hand	10	5	5	20
2.	Butcher's knife	10	5	5	20
3.	Chopping board	5	10	5	20
Total		25	20	15	60

Processing of the meat samples

Sample preparation

Approximately 10 g meat sample was triturated using sterile pestle and mortar by addition of 90 ml NSS (1:10 dilution) to have homogenate mixture. Ten ml of the sample homogenate was mixed with 90 ml of MacConkey Broth followed by incubation at 37 °C for 24 hr for enrichment. The enriched samples were streaked on MacConkey agar plates and incubated at 37 °C for 24 hr. Subsequently, the Plates with pink colour colonies were selected and re-inoculated on Eosin Methylene Blue (EMB) agar plate and incubated at 37ºC for 24 hr. The colonies with green metallic sheen were picked up and stored on Nutrient agar slant for further studies.

Screening biochemical tests

The sparse colony from the EMB plates were selected and stabbed/streaked on Triple Sugar Iron (TSI) agar and Lysine Iron Agar (LIA) and incubated at 37ºC for 18 hr and reaction was noted. Based on standard colony morphology and how they responded to TSI and LIA agar, the growth was firstly subjected to Gram's staining followed by to catalase, oxidase and IMViC tests. The isolates which were catalase positive, oxidase negative and IMViC pattern as: +/+/-/-, were presumed to be *E. coli*, and were preserved for further studies.

Confirmation by PCR

DNA Template preparation by boiling and snap chilling method

A microfuge tube (1.5 ml) contained 100 µl of sterile milli Q water was added with 2-3 colonies of an overnight-grown *E. coli* culture from MacConkey agar plates, and the suspension was heated for 10 min in a boiling water. The microfuge tube was immediately placed on ice, and centrifuged at 8000 rpm for 5 minutes at 4 °C. The supernatant was utilized as a template to detect the *E. coli* by PCR.

Screening for *E. coli* by PCR

The isolates were screened for *E. coli* by targeting the allergen as oligonucleotide sequence mention in Table 3, as per the standard protocol described in the literature reviewed.

Table 3: Primer used for detection *E. coli*

Target gene	Oligonucleotide sequence (5′ → 3′)	Amplicon length	Reference
alr	**F:** CTGGAAGAGGCTAGCCTGGACGAG	369 bp	Yokoigawa *et al*. (1999) [30], Hegde *et al.* (2013) [10]
	R: AAAATCGCCACCGGTGGAGCGATC		

The PCR for amplification of the *E. coli* was set up in 25 µl reaction mixture. Following initial trials with varying concentrations of components, the reaction mixture was optimized as per Table 4 and the thermal cycling condition for identification as mention in Table 5.

Table 4: Concentration of various components of reaction mixture

Sr. No.	Components	Quantity Final	Concentration
1	DNase-RNase free water	5.5 µl	--
2	2X PCR master mix	12.50 µl	2X
3	Forward Primer (Stock cont^{n}:100 pmol/µl)	1µl	10 pmol
4	Reverse Primer (Stock cont^{n}:100 pmol/µl)	1 µl	10 pmol
5	DNA Template	5.00 µl	--
Grand Total		25.00 µl	--

Table 5: Thermal cycling condition for identification of *E. coli* by PCR

Target gene	Simplex PCR for *E. coli*				
	Initial denaturation	Denaturation	Annealing	Extension	Final extension
alr	94 ºC for 5 minutes	94 ºC for 30 seconds	56 ºC for 45 seconds	72 ºC for 45 seconds	72 ºC for 10 minutes
		Repeated for 30 cycles			

Results and Discussion

Isolation and Identification of *E. coli*

The standard bacteriological analysis of total 270 samples (210 raw meat and 60 swab) following standard protocol, which in turn yielded total 63 (23.33%) *E. coli* isolates, which comprised of 51/210(24.28%) from meat and 12/60 (20%) from swab samples.

Occurrence of *E. coli* in meat

The occurrence of *E. coli* in meat samples of different animals and swab samples by cultural method is given Table 6. Out of 270 samples 63 (23.33%) samples were found to be positive for *E. coli* which comprised of 51/210(24.28%) from meat and 12/60 (20%) from swab samples.

Table 6: Occurrence of *E. coli* in the meat samples

Sr. No.	Type of meat	Number examined	Number positive	Occurrence (%)
1.	Chicken	60	14	23
2.	Chevon	50	11	22
3.	Mutton	50	08	16
4.	Carabeef	50	18	36
Total		210	51	24.28

Present work noticed 36% occurrence of the pathogen in Carabeef, which is in agreement with the findings of Hussien *et al.* (2019) [4] who observed the higher prevalence rates of *E. coli* from 0.01 to 43.4%.

In the present study *E. coli* showed the higher occurrence in the Carabeef (36%), followed by the chicken (23%). Adesiji *et al.* (2011) [2] also reported higher occurrence of 48% and 16%, respectively. However, Uddin *et al.* (2018) [11] reported 20.6% occurrence of *E. coli* in the poultry meat samples.

The occurrence of the bacteria in the chevon samples, in the present study was 22% which in close proximate to the findings of Kumar *et al.* (2022) [5], who reported 24% prevalence. Also fall in between the range of the prevalence in chevon from 16.66% to 33.33% noticed by Rathod *et al.* (2004) [7]. Though, Sumitha *et al.* (2016) [9] isolated *E. coli* in 18% chevon samples which showed lower rate of contamination. However, it is in contrast to the findings of Adesiji *et al.* (2011) [2], who could not isolate this pathogen from 75 chevon samples, expressing extra ordinary hygienic practice might be followed at the place.

The 16% mutton samples in the present study contained *E. coli* which is similar to findings of Kumar *et al.* (2022) [5] who obtained the prevalence of 16% in the chevon.

Odds ratio and Relative risk profile for occurrence of *E. coli* in the meat samples: Odds ratio is a statistical measure which describes the association between two events. In the current investigation, if mutton is treated as 1 at 95% confidence and odds ratio is greater than 1, it implies that the exposed group has a higher chance of contacting the infection versus the non-exposed group.

In the present study Chicken, Chevon and Carabeef have the odd's ratio greater than 1, therefore, the relative risk of getting infection by consumption of Chicken is 1.45 times, Chevon is 1.37 times and Carabeef is 2.25 times higher than the Mutton. The relative risk of getting infection is in descending order of Carabeef, followed by Chicken and Chevon, as mentioned in Table 7.

Table 7: Odds Ratio and Relative Risk profile of occurrence of *E. coli* in the meat samples

S. No.	Type of meat	Odds Ratio		Risk Ratio (Relative Risk)	
		Value	95% CI*	Value	95% CI*
1.	Chicken	1.5978	0.6093-4.1904	1.4583	0.6662-3.1922
2.	Chevon	1.4808	0.5395-4.0642	1.375	0.6044-3.1284
3.	Mutton	Reference category (Considered as 1)			
3.	Carabeef	2.9531	1.1405-7.6465	2.25	1.0791-4.6915

* CI = Confidence Interval (Lower limit - Upper limit)

As mentioned in the Table 8, higher level of contamination of *E. coli* was 25% found on Butcher's knives and Chopping board, while lower level of 10% evident on Butcher's hands.

Table 8: Occurrence of *E. coli* in the swab samples

S. No.	Type of swab	Number of samples	Number of Isolates	Occurrence (%)
1.	Butcher's hands	20	2	10.00
2.	Butcher's knives	20	5	25.00
3.	Chopping board	20	5	25.00
Total		60	12	20

Odds Ratio and Relative Risk profile for occurrence of *E. coli* isolated from swab samples

In the current investigation, if Butcher's hand is treated as 1 and Odds Ratio expressed greater than 1, it implies that the exposed group has higher chance of contacting the infection of *E. coli* versus the non-exposed group. Butcher's knives and Chopping board swabs have Odds Ratio 2.5, indicating 2.5 times higher risk of propagating infection through them, as revealed from Table 9.

Table 9: Odds Ratio and Relative Risk profile for occurrence of *E. coli* isolated from swab samples

Type of meat	Odds Ratio		Risk Ratio (REL Risk)	
	Value	95% CI*	Value	95% CI*
Butcher's hand	Reference category (Considered as 1)			
Butcher's knife	3.00	0.5073-17.7409	2.5	0.5478-11.4101
Chopping board	3.00	0.5073-17.7409	2.5	0.5478-11.4101

* CI = Confidence Intervals (Lower limit - Upper limit)

Table 10: Over all occurrence of *E. coli* in the meat samples

SN	Type of sample	Number of samples	Number Positive	Occurrence (%)
1	Meat	210	51	24.28
2	Swab samples	60	12	20.00
3	Total	270	63	23.33

The overall occurrence in the present study was 23.33%.

Table 11: Odds ratio and Relative risk profile for overall occurrence of *E. coli*

Type of meat	Odds Ratio		Risk Ratio (Relative Risk)	
	Value	95% CI*	Value	95% CI*
Meat	1.283	0.6328-2.6015	1.2143	0.6939-2.1249
Swab samples	Reference category (Considered as 1)			

* CI = Confidence Intervals (Lower limit - Upper limit)

Statistical analysis

To investigate the relationship between the presence and absence of *E. coli* in the type of meat, type of swab and overall occurrence, the Chi-Square test was performed.

The results revealed there was no association between the presence and absence *E. coli* and the type of meat ($\chi^2 = 5.770$, p = 0.128; Fischer's exact value = 5.508, p = 0.143), as well as the type of swab ($\chi^2 = 1.875$, p = 0.552; Fischer's exact value = 1.941, p = 0.444), as shown in Table 12 and 13.

The results of analysis between the presence and absence *E. coli* in the presence both meat and swab revealed there was no dependence on each other ($\chi^2 = 0.479$, p = 0.604) as given in Table 14.

Table 12: Association between types of meat sample and occurrence of *E. coli*

Type of Meat	No. of Samples	Negative sample	Positive sample	Chi-Square Tests			
				Pearson Chi-Square		Fisher's Exact Test	
				Value	p	Value	p
Chicken	60	46 (76.70%)	14 23.30%	5.77	0.128 (NS)	5.508	0.143 (NS)
Chevon	50	39 (78.00%)	11 22.00%				
Mutton	50	42 (84.00%)	8 16.00%				
Carabeef	50	32 (64.00%)	18 36.00%				
Total	210	159 (75.70%)	51 (23.30%)				

Table 13: Association between types of swabs and occurrence of *E. coli*

S. No.	Type of swab	No. of Samples	Negative sample	Positive sample	Chi-Square Tests			
					Pearson Chi-Square		Fisher's Exact Test	
					Value	p	Value	p
1.	Butcher's hand	20	18 (90.00%)	2 (10.00%)	1.875	0.552 (NS)	1.941	0.444 (NS)
2.	Butcher's knife	20	15 (75.00%)	5 (25.00%)				
3.	Chopping board	20	15 (75.00%)	5 (25.00%)				
Total		60	48 (80.00%)	12 (20.00%)				

Table 14: Association between the type of samples and presence of *E. coli*

S. No.	Type of sample	No. of samples	No. of Negative Samples	No. of Positive samples	Chi-Square Tests			
					Pearson Chi-Square		Fisher's Exact Test	
					Value	p	Value	p
1.	Meat	210	159 (75.71%)	51 (24.28%)	0.479	0.604 (NS)	-	-
2.	Swab samples	60	48 (80.00%)	12 (20.00%)				
3.	Total	270	207 (76.66%)	63 (23.33%)				

Conclusions

A total of 270 samples were investigated, which included 210 meat samples (60 samples of chicken and 50 each, of chevon, mutton and carabeef) and 60 swab samples (20 samples each of butcher's knives, hands and chopping board) gave 63(23.3%) *E. coli*. Out of 210 meat samples 51 (24.28%) were positive for *E. coli.* The species wise spread of isolates showed that 14/60 (23%), 11/50 (22%), 8/50 (16%) and 18/50 (36%) samples were positive, respectively in case of chicken, chevon, mutton and carabeef. Of 60 swab samples which includes 20 samples each of butcher's knife, hand and chopping board, 12 (20%) yielded *E. coli.* The positive samples included 2 (10%) swab samples from butcher's hand and 5 (25%) each, of butcher's knife and chopping board were positive. The relative risk of getting infection among the meat samples is in descending order of Carabeef, followed by Chicken and Chevon. The swab samples from butcher's knives and chopping board swabs have higher risk of propagating infection through them. Among both the meat and swab samples the risk of getting the infection is more

from the meat samples. The statistical results revealed there was no association between the presence and absence *E. coli* and the type of meat, swab and overall occurrence.

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