The Pharma Innovation Journal 2023; SP-12(9): 2498-2507

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(9): 2498-2507

www.thepharmajournal.com
Received: 01-07-2023
Accepted: 07-08-2023

Navya Sri Bairi
Department of Veterinary Public Health and Epidemiology, College of Veterinary science & Animal Husbandry, Kamdhenu University, Navsari, Gujarat. India

CV Savalia
Department of Veterinary Public Health and Epidemiology, College of Veterinary science & Animal Husbandry, Kamdhenu University, Navsari, Gujarat. India

R Kumar
Department of Veterinary Public Health and Epidemiology, College of Veterinary science & Animal Husbandry, Kamdhenu University, Navsari, Gujarat. India

IH Kalyani
Department of Veterinary Microbiology, College of Veterinary science & Animal Husbandry, Kamdhenu University, Navsari, Gujarat. India

Corresponding Author:
R Kumar
Department of Veterinary Public Health and Epidemiology, College of Veterinary science & Animal Husbandry, Kamdhenu University, Navsari, Gujarat. India

Isolation and identification of *Escherichia coli* from different varieties of meat and meat handlers

Navya Sri Bairi, CV Savalia, R Kumar and IH Kalyani

Abstract
The present research work was carried out in the city of Navsari, Gujarat State, with the aim to isolate and identify *Escherichia coli* from meat of various species of animals using standard isolation methods followed by confirmation with PCR as well judging antibiotic sensitivity pattern of the isolates. During the study a total of 270 samples were processed, which included 210 meat samples (comprised of 60 samples of chicken, and 50 samples each, of chevon, mutton and carabeef) as well as 60 swab samples (20 samples each, of butcher's knives, butchers' hands and meat chopping board). Among 270 samples 63(23.3%) yielded growth of *E. coli*; which included 51(24.28%) from meat and 12(20%) from swab samples. The meat variety-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. Antibiotic sensitivity test of all 63 isolates was performed using 11 antibiotics by agar disc diffusion method. The isolates exhibited 100% resistance towards Penicillin followed in descending order by Erythromycin (93.7%), Streptomycin (85.71%), Amikacin (77.7%) and Gentamicin (63.49%). An intermediate sensitivity/resistance was noticed against Amoxicillin (69.8%). While, the highest sensitivity of 92.5% was reported to Tetracycline followed by 84.12% against each, Norfloxacin and Ciprofloxacin, 82.5% to Chloramphenicol and, Enrofloxacin showed the least sensitivity of 68.25%.

Keywords: *E. coli*, meat, antibiotic sensitivity pattern, PCR, swab

Introduction
Meat is a rich source of protein as well as other nutrients which makes it an ideal medium for the growth and proliferation of various bacteria resulting in spoilage of meat tends to food borne illness in the consumers. Lack of sanitation, poor post-production storage, careless handling of food items, etc. also leads to meat contamination. Uncooked meat found to harbor numerous bacterial species including *Escherichia coli* (*E. coli*), *Enterobacter aerogens*, *Salmonella* spp., *Staphylococcus* spp., *Pseudomonas* spp., etc. The zoonotic nature of *E. coli* infections can lead to severe food poisoning in humans. (Thanigaivel and Anandhan, 2015) [25]. *Escherichia coli* is responsible for a vast range of diseases in both humans and animals, globally. Pathogenic *E. coli* strains are divided into two groups: extra intestinal pathogenic *E. coli* (EPEC) and intestinal pathogenic *E. coli*. Septicemia, endovascular infections, deep surgical wound infections, pneumonia, newborn meningitis, bacteremia, and urinary tract infections are among the illnesses brought on by EPEC strains. (Russo and Johnson, 2000) [18].
The six pathotypes of intestinal pathogenic *E. coli* are: Diffusely Adherent *E. coli* (DAEC), Enteroaggregative *E. coli* (EAggEC), Enteropathogenic *E. coli* (EPEC), Enteroinvasive E. *coli* (EIEC), and Verocytotoxigenic *E. coli* (VTEC) (Torres *et al*,2010) [26].
The VTEC, sometimes referred to as lethal shiga toxin producing *E. coli* (STEC), which is a pathotype of *E. coli* linked to human food-borne illnesses. Hemolytic Uremic Syndrome (HUS), Hemorrhagic Colitis (HC), Thrombotic Thrombocytopenic Purpura (TTP), acute renal failure, microangiopathic anaemia, and haemolytic anaemia are serious gastrointestinal disorders caused by it that have a considerable impact on public health globally. *E. coli* O157:H7, one of several serologically different strains of VTEC, is the common cause of VTEC infections. (Hussien *et al*, 2019) [11].

Material 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.

Sample collection

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

Sample processing

Preparation of meat and swab samples

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 in flask containing 90 ml of MacConkey Broth and swab samples were inoculated in tubes containing 10 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 37 °C 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 37 °C 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 of *E. coli* 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 *alr* gene 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	94ºC for 5 minutes	94ºC for 30 seconds	56 ºC for 45 seconds	72 ºC for 45 seconds	72 ºC for 10 minutes
		Repeated for 30 cycles			

Antibiotic Susceptibility test

The isolates were subjected to antibiotic sensitivity test as per the guidelines of CLSI, 2017 [8].

Selection of antibiotics

For this study 11 antibiotics were selected based on their mechanism of action *viz*. Cell wall synthesis inhibitors: Amoxicillin and Penicillin; Protein synthesis inhibitors: Amikacin, Chloramphenicol, Erythromycin, Gentamicin, Streptomycin and Tetracycline; and DNA gyrase inhibitors: Ciprofloxacin, Norfloxacin and Enrofloxacin. The details of antibiotic discs used is given in Table 6.

Table 6: Details of antibiotic discs used

Sr. No.	Mechanism of Action	Antibiotic disc	Symbol	Concentration (mcg)
1	Cell wall synthesis inhibitors	Amoxicillin	AMX	30
2		Penicillin	P	10 units
3	Protein synthesis inhibitors	Amikacin	AK	30
4		Chloramphenicol	C	30
5		Erythromycin	E	15
6		Gentamicin	GEN	10
7		Streptomycin	S	10
8		Tetracycline	TE	10
9	DNA gyrase inhibitors	Ciprofloxacin	CIP	5
10		Enrofloxacin	EX	10
11		Norfloxacin	NX	5

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.

Culture media

For isolation of *E. coli* Mac Conkey broth was used as enrichment medium, followed by culturing on Mac Conkey agar and Eosin Methylene Blue agar as selective and differential medium, respectively. The samples were enriched in Mac conkey broth (Fig 1), followed by plating on the Mac Conkey Agar. The pink color colonies (Fig 2) from selective medium were selected and streaked further on EMB agar, where colonies showing greenish metallic sheen (Fig3) were further confirmed as *E. coli* by further analysis.

Fig 1: Turbidity in the enrichment of raw meat sample in Mac conkey broth

Fig: 2 Pink colored colonies on Mac conkey agar plate

Fig 3: Greenish metallic sheen colonies on EMB agar plate

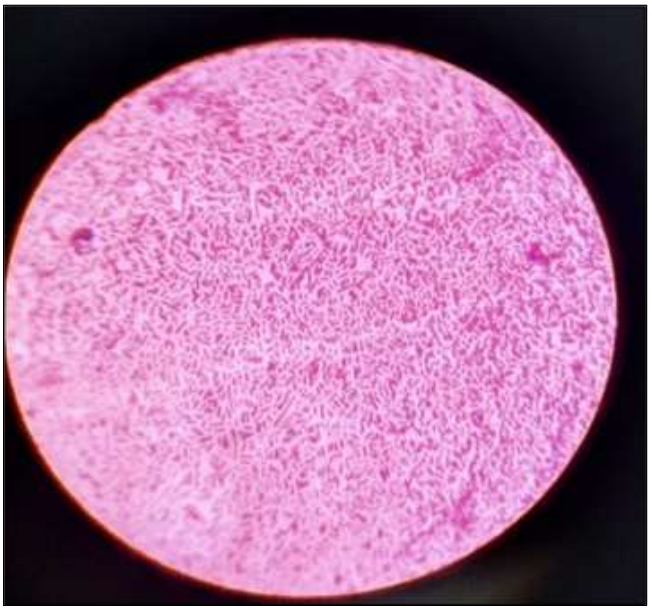


Fig 4: Gram negative rods under 100x light microscope

Phenotypic and biochemical confirmation

The suspected colonies grown on EMB agar were further subjected to biochemical tests. All the 63 isolates were Gram negative rods (Fig4), the isolates displayed acidic/acidic, H_2S negative (-) and were positive for gas production on the Triple Sugar Iron (TSI) (Fig 5). The isolates cultured on LIA showed alkaline/alkaline, H_2S negative (-) and gas negative (-) (Fig 6). They were motile, as expressed on the Mannitol motility medium (Fig 7). All the isolates produced Indole, marked with the formation of a red/pink ring at the top (Fig 8). The Methyl Red test was positive with the red color formation in the test tube (Fig. 9). The Vogues Proskauer test (Fig 10) and Citrate utilization test (Fig 11) were negative by the all isolates except 1(P-16) isolate which utilized citrate. A catalase positive test was indicated by bubble formation (Fig 12) and the Urease (Fig 13) and oxidase tests (Fig 14) were negative, indicated by 'no change in colour'. The nitrate test was positive is indicated by pink colour formation (Fig 15). All 63 isolates fermented lactose, glucose and mannitol, expressed by pink color development; but did not ferment inositol, mannose and maltose, marked as 'no colour change' (Fig 16). The isolates did not hydrolyze gelatin and starch (Fig 17 and Fig 18). In the present work, all 63 isolates expressed the biochemical characters as described by the Edward and Ewing, 1972 [9].

Fig 5: Triple sugar iron agar
A: Negative control
B: Colour change from read to yellow and gas formation is positive for *E. coli*

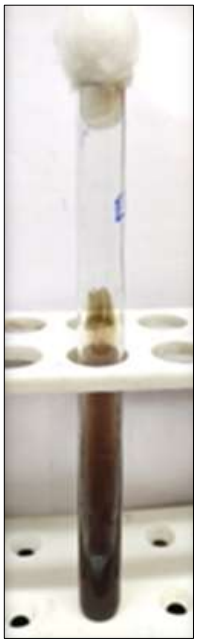


Fig 6: Lysine iron agar: alkaline slant and butt

Fig 7: Mannitol motility test positive

Fig 8: Indole ring test
A: Negative control
B: Positive-pink colour ring formation for *E. coli*

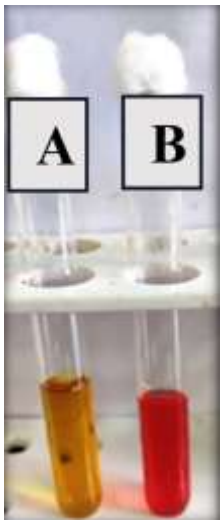
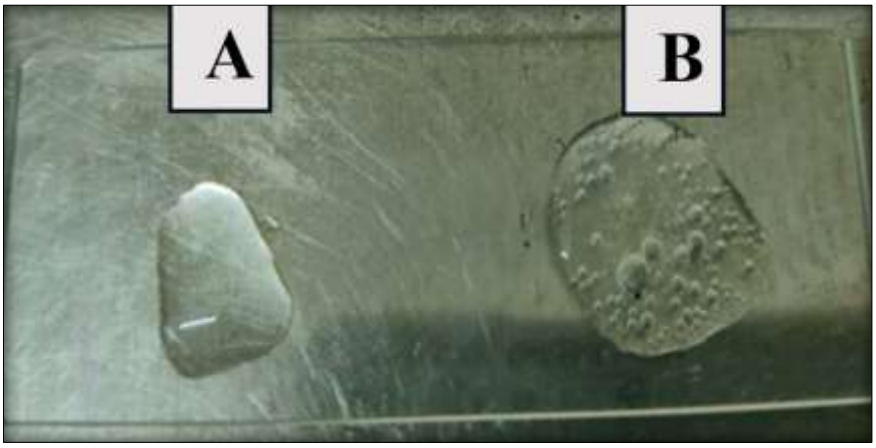


Fig 9: Methyl Red test
A: Negative control
B: Positive-change in colour to red for *E. coli*

Fig 10: Vogues-Proskauer test
A: Negative-no change in colour for *E. coli*
B: Positive control

Fig 11: Citrate test
A: Negative - no change in colour for *E. coli*
B: Positive control



A: Negative control
B: Positive -bubble formation for *E. coli*

Fig 12: Catalase test

Fig 13: Urease test negative for *E. coli*

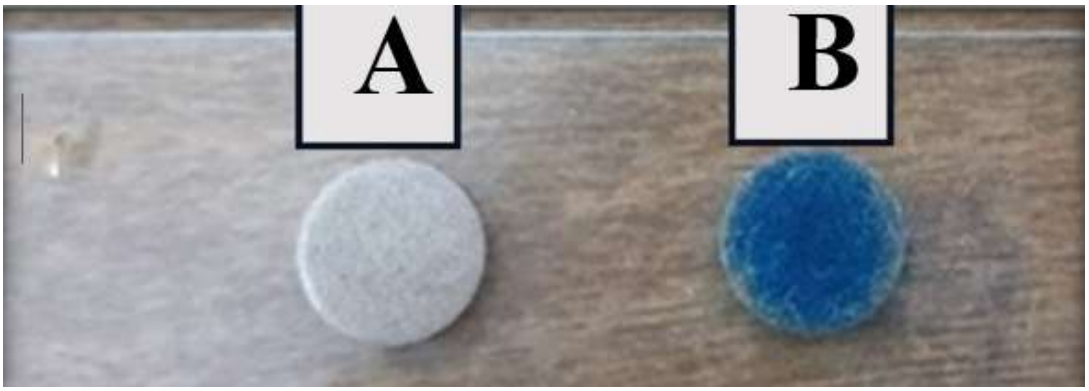


Fig 14: oxidase test
A: Negative - no colour change for *E. coli*
B: Positive control

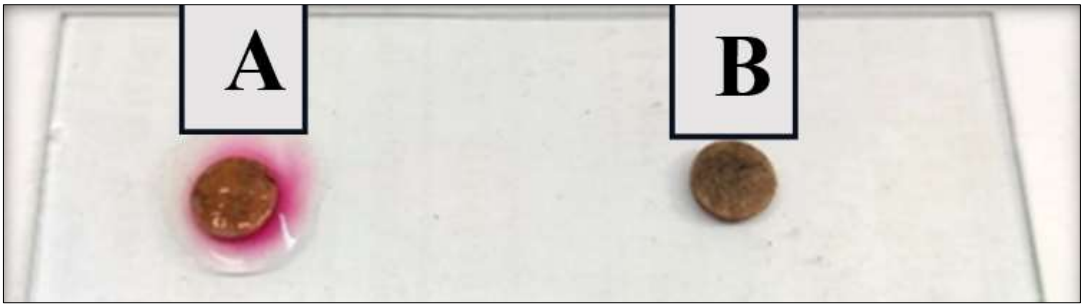


Fig 15: Nitrate test
A: Positive-pink colour formation for *E. coli*
B: Negative control

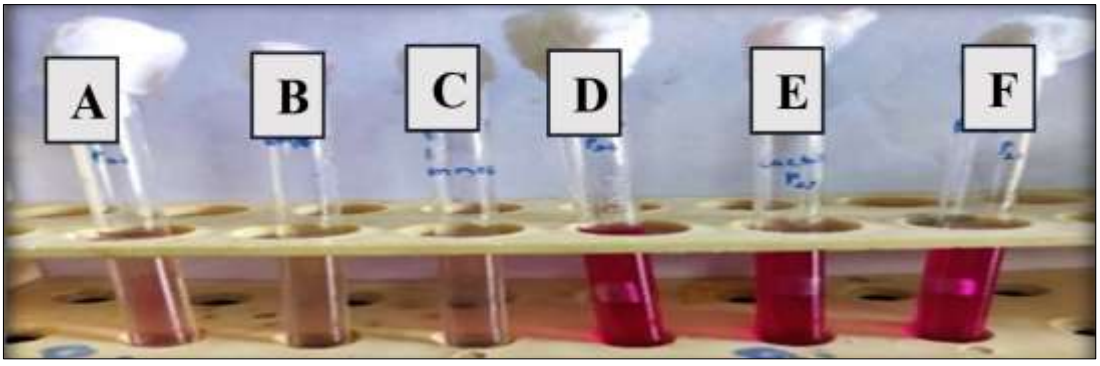


Fig 16: Sugar fermentation test (change in colour indicates positive reaction)
A: Inositol(-ve), **B**: Maltose (-ve), **C**: Mannose(-ve),
D: Lactose (+ve), **E**: Glucose (+ve), **F**: Mannitol (+ve)

Fig 17: Gelatin hydrolysis test
A: Negative gelatin hydrolysis for *E. coli*
B: Positive control

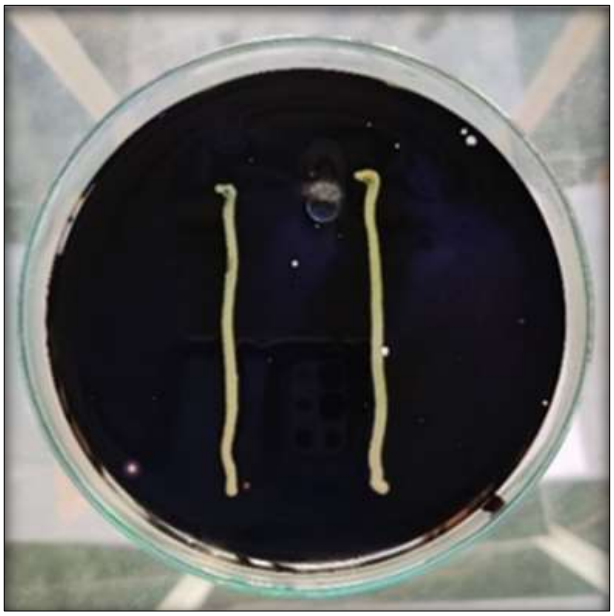


Fig 18: Starch hydrolysis test negative for *E. coli*

Barrow and Feltham (1993) [5] identified that 0-15% of *E. coli* were able to utilize citrate. In the present study atypical or unusual biochemical character was observed in 1 isolate from chicken sample (1.66%) which utilized the citrate similar result of 2.34% was reported by Mishra *et al* (2002) [15] and Smrati *et al* (2000) [23].

Occurrence of *E. coli* in meat and meat handlers

The occurrence of *E. coli* in meat samples of different animals and swab samples by cultural method is given Table 7 and Table 8. 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 7: 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

It is evident from Table 7 that maximum level of contamination with *E. coli* was noticed in Carabeef, followed in descending order by chicken (23%), Chevon (22%) and Mutton (16%), with overall occurrence of 24.28 per cent in the meat samples.

Present work noticed 36% occurrence of the pathogen in Carabeef, which is in agreement with the findings of Hussien *et al* (2019) [11] who observed *E. coli* from 0.01 to 43.4% in the meat samples they studied.

In the present study *E. coli* showed higher occurrence in the Carabeef (36%), followed by the chicken (23%). Adesiji *et al* (2011) [1] also reported higher occurrence of 48% and 16%, respectively. However, Uddin *et al* (2018) [27] reported 20.6% occurrence of *E. coli* in the poultry meat samples, which slight lower.

Looking to the chevon, in the present study was 22% samples contained *E.coli*, which is in close proximate to the findings of Kumar *et al* (2022) [13], who reported 24% recovery from chevon. Also it fall in between the range of the prevalence in chevon from 16.66% to 33.33% noticed by Rathod *et al* (2004) [17]. Though, Sumitha *et al* (2016) [24] reported *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) [1], who could not isolate this pathogen from 75 chevon samples, expressing extra ordinary hygienic practice might be followed at the place of their study.

The 16% mutton samples in the present study contained *E. coli* which is similar to findings of Kumar *et al* (2022) [13] who obtained the prevalence of 16% in the chevon.

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

Sr. 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

As mentioned in the Table 8, higher level of contamination (25%) of *E. coli* was found on Butcher's knives and Chopping board, while lower level (10%) was evident on Butcher's hands. Ajay (2018) [2] found similar level of contamination on knife swab samples (25%), but contrary to the findings of chopping board (47.83%), indicating greater risk of propagation of *E. coli* from the chopping board.

Table 9: Over all occurrence based on type of the sample

SN	Nature of sample	Samples tested	Samples positive	% value
1	Minced meat	90	24	26.60
2	Breast/Rib	50	11	22.20
3	Thigh muscle	35	06	17.14
4	Wing	05	03	60.00
5	Giblet/Pluck	30	07	23.30
Total		210	51	24.28

The contamination of *E. coli* in Minced meat, Breast/Rib, Thigh muscle, Wing and Giblet/Pluck was 26.6%, 22.2%, 17.14%, 60% and23.3%, respectively, as mentioned Table 9.

Molecular detection of *E. coli* by PCR

The traditional diagnosis that rely on pathogen phenotype rather than genotype are gradually being replaced by rapid molecular techniques. The PCR has increasingly been referred for detecting particular bacteria. The PCR, surpassed the probe and signal amplification methods as the most popular nucleic acid amplification technology for diagnosing infectious diseases.

The isolates which were confirmed by cultural and biochemical tests were further subjected to PCR. The DNA was extracted using snap chilling and quality of DNA is analyzed by gel electrophoresis which did not show any shearing was considered as pure. It was subjected to PCR following the standard protocols.

Lane M: 100 bp ladder, Lane PC: Positive control (*E. coli* ATCC 25922), Lane NC: Negative control (*S. aureus* ATCC 25923), Lane 1-7: Samples

Fig 19: Representative agarose gel showing PCR amplified product of 369bp for *alr* gene of *E. coli* isolates

The isolates were screened for *E. coli* by targeting the *alr* and all 63 isolates of *E. coli* and yielded 369 amplicons (Hegde *et al*, 2013) [10] as shown in Fig 19.

Antibiotic Susceptibility

As the results summarized Table 10, Fig 20 and Fig 21, all the 63 isolates of *E. coli* were cent percent resistant to Penicillin, followed in descending order by Erythromycin (93.7%), Streptomycin (85.71%), Amikacin (77.7%) and Gentamicin (63.49%). Whereas, all the isolates were 92.5% sensitive to Tetracycline, followed by Ciprofloxacin and Norfloxacin (84.12%) each, Chloramphenicol (82.5%) and Enrofloxacin (68.25%). Intermediate resistance was exerted in case of Amoxicillin (69.2%).

Table 10: Summary of the Antibiotic susceptibility test

SN	Antibiotics	S%	IM%	R%
1	Penicillin	-	-	100
2	Amoxicillin	-	69.8	30.15
3	Ciprofloxacin	84.12	11.11	4.7
4	Enrofloxacin	68.25	4.76	26.6
5	Norfloxacin	84.12	-	15.8
6	Amikacin	11.11	11.11	77.77
7	Chloramphenicol	82.5	9.5	7.9
8	Erythromycin	-	6.3	93.7
9	Gentamicin	26.98	9.5	63.49
10	Streptomycin	-	14.28	85.71
11	Tetracycline	92.5	3.17	4.76

Fig 20: Anti-microbial susceptibility patterns of *E. coli* showing zones of inhibition of Amoxicillin, Norfloxacin, Chloramphenicol, Penicillin, Ciprofloxacin, Erythromycin

Fig 21: Anti-microbial susceptibility patterns of *E. coli* showing zones of inhibition of Gentamicin, Amikacin, Streptomycin, Tetracycline, Enrofloxacin

Looking to the present findings of Penicillin, results were in accordance with the results of Chakravarty *et al* (2015) [6], Sabir *et al* (2014) [19], Nontongana *et al* (2014) [16], However, the results of present study were contrary to the findings of Chandrasekaran *et al* (2014) [7] and Jeyasanta *et al* (2012) [12] who reported 63% and 82.41% of resistance to Penicillin, respectively. The Tetracycline showed highest sensitivity similar to the results of Van den Bogaard *et al* (2001) [28], Singh *et al* (1992) [22], Manna *et al* (2006) [14], Sharma *et al* (2017) [21] and Yadav *et al* (2007) [29] while highest resistance was shown by Akond *et al* (2009) [3], Sharada and Ruban (2010) [20] and Aksoy *et al* (2007) [4].

Conclusions

Out of 210 meat samples 51 (24.28%) were positive for *E. coli.* The meat species-wise distribution of isolates showed that 14/60 (23%), 11/50 (22%), 8/50 (16%) and 18/50 (36%) chicken, chevon, mutton and carabeef samples were positive, respectively. Of 60 swab samples which includes 20 samples each of butcher's knife, butcher's hands and chopping board, 12 (20%) yielded *E. coli.* The positive samples included 2 (10%) swab samples from butcher's hands and 5 (25%) each, of butcher's knives and chopping boards. Observing meat type-wise distribution of 51 isolates of *E. coli*, 24 (26.6%), 11 (22.22%), 6 (17.14%), 3 (60%) and 7 (23.3%) were obtained from minced meat, breast/rib muscle, thigh muscle, wing and giblet/pluck, respectively. The higher occurrence was observed in minced meat and the least in the thigh muscle. The 20 swab samples each, of the Butcher's hands, knives and chopping board were assessed. The occurrence of *E. coli* was 20% (12/60) in different swab samples including 2 (10%) from Butcher's hands, and 5 (25%) each, from knives and chopping board respectively. On preliminary biochemical examination all 62 but one isolates showed characteristic IMViC pattern, and 1 isolate from chicken was Citrate positive. The antibiogram studies revealed the most effective antibiotic agents against *E. coli* was Tetracycline which showed 92.5% sensitivity, followed by Ciprofloxacin and Norfloxacin which showed equal magnitude of 84.12% sensitivity. All the *E. coli* isolates exhibited cent percent resistance against Penicillin.

Acknowledgement

The authors acknowledge the support rendered by the Dean, College of Veterinary science & Animal Husbandry, Navsari -396450 and Director of Research & Dean, PGS, Kamdhenu University, Gandhinagar - 382010, Gujarat, India, for providing necessary facilities and funds to carry out this work.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Adesiji YO, Alli OT, Adekanle MA, Jolayemi JB. Prevalence of *Arcobacter*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* species in retail raw chicken, pork, beef and goat meat in Osogbo, Nigeria. Sierra Leone J. of Biomed Research. 2010;3(1):8-12.
2. Ajay BT. Mitigation of Enterohaemorrhagic *Escherichia coli* on meat contact surfaces using Phytochemicals. [M.V.Sc. Dissertation, submitted to Kerala Veterinary and Animal Sciences University]. 2018. Wayanad.
3. Akond MA, Alam S, Hassan SMR, Shirin M. Antibiotic resistance of *Escherichia coli* isolated from poultry and poultry environment of Bangladesh. Int. J. of Food Safety. 2009;11(2):19-23.
4. Aksoy A, Yildirim M, Kacmaz B, Apan TZ, Goçmen JS. Verotoxin production in strains of *Escherichia coli* isolated from cattle and sheep, and their resistance to antibiotics. Turkish J. of Vet and Animal Sci. 2007;31(4):225-231.
5. Barrow GI, Feltham RKA. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd edition, 1993, 140-43, Cambridge University Press, Cambridge. http://dx.doi.org/10.1017/CBO9780511527104
6. Chakravarty MS, Ganesh PRC, Amaranth D, Shanthi Sudha B, Subhashini M. *Escherichia coli*-occurrence in the meat of shrimp, fish, chicken and mutton and its antibiotic resistance. European J. of Exp Bio. 2015;5(7):41-48.
7. Chandrasekaran D, Venkatesan P, Tirumurugaan KG, Nambi AP, Thirunavukkarasu PS, Kumanan K, *et al* Pattern of antibiotic resistant mastitis in dairy cows. Vet World. 2015;7(6):389-394.
8. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute;

counts in raw milk. Dairy, Food and Env. Sanitation. 2017;20(8);606-611.
9. Edwards PR, Ewing W. Identification of Enterobacteriaceae 4th edition, Elsvier Sci. Burgees Publishing Co., USA; c1972.
10. Hegde R, Isloor S, Prabhu KN, Shome BR, Rathnamma D, Suryanarayana VVS, *et al* Incidence of subclinical mastitis and prevalence of major mastitis pathogens in organized farms and unorganized sectors. Indian J. of Microbio. 2013;5;315-320.
11. Hussien H, Elbehiry A, Saad M, Hadad G, Moussa I, Dawoud T, *et al* Molecular characterization of *Escherichia coli* isolated from cheese and biocontrol of Shiga toxigenic *E. coli* with essential oils. Italian J. of Food Safety. 2019;8(3):162-167.
12. Jeyasanta KI, Aiyamperumal V. Patterson Prevalence of antibiotic resistant *Escherichia coli* in sea foods of Tuticorin coast, Southeastern India. Advanced Bio. Research. 2012;6(2):70-77.
13. Kumar K, Sharma NS, Kaur P, Arora AK. Molecular detection of antimicrobial resistance genes and virulence genes in *E. coli* isolated from sheep and goat faecal samples. Indian J of Ani. Research. 2022;56(2):208-214.
14. Manna SK, Brahmane MP, Das R, Chandana M, Batabyal S. Detection of *Escherichia coli* O157 in foods of animal origin by culture and multiplex polymerase chain reaction. J. of Food Science and Tech. 2006;43(1):77-79.
15. Mishra A, Sharda R, Chhabra D, Moghe MN. *Escherichia coli* isolates from domestic poultry. Indian J. of Ani. Sci. 2002;79:727-729.
16. Nontongana N, Sibanda T, Ngwenya E, Okoh AI. Prevalence and antibiogram profiling of *Escherichia* coli pathotypes isolated from the Kat River and the Fort Beaufort abstraction water. Int. J. of Env. Research and Public Health. 2014;11(8):8213-8227.
17. Rathod KS, Ambedkar RK, Zanjad PN, Deshmukh VV, Raziuddin M. Microbiological quality of chevon sold in Parbhani City. J. of Vet. Public Health. 2004;2(1/2):19-22.
18. Russo TA, Johnson JR. Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *Escherichia coli*: ExPEC. J. of Inf. Dis. 2000;181(5):1753-1754.
19. Sabir S, Anjum AA, Ijaz T, Ali MA, Nawaz M. Isolation and antibiotic susceptibility of *E. coli* from urinary tract infections in a tertiary care hospital. Pakistan J. of Med. Sci. 2014;30(2): 389.
20. Sharada R, Ruban SW. Isolation, characterization and antibiotic resistance pattern of *Escherichia coli* isolated from poultry. American-Eurasian J. of Sci. Research. 2010;5(1):18-22.
21. Sharma B, Verma AK, Jain U, Yadav JK, Singh R, Mishra R. Occurrence of multidrug resistant *Escherichia* coli in ground water of Brij region (Uttar Pradesh) and its public health implications. Vet. World. 2017;10(3):293.
22. Singh M, Chaudhry MA, Yadava JNS, Sanyal SC. The spectrum of antibiotic resistance in human and veterinary isolates of *Escherichia* coli collected from 1984-86 in northern India. J. of Antimicrobial Chemotherapy. 1992;29(2):159-168.
23. Smrati D, Rakesh S, Daljeet C, Varsha S, Reddy AG, Rajesh S. Enterotoxigenic *Escherichia* coli strains isolated from diarroheic goats. Indian J. of Comparative Microbiol. Immunol and Inf. Diseases. 2000;21(1):68-69.
24. Sumitha P, Sukumar K, Eswaran MA. Assessment of microbial quality of fresh chevon sold in Namakkal district of Tamil Nadu. Indian Vet. J. 2016;93(11):80-82.
25. Thanigaivel G, Anandhan AS. Isolation and characterization of microorganisms from raw meat obtained from different market-places in and around Chennai. J. of Pharmaceutical, Chemical and Biological Sci. 2015;3(2):295-301.
26. Torres AG, Arenas-Hernandez MM, Martínez-Laguna Y. Overview of *Escherichia coli*. Pathogenic *E. coli* in latin America. 2010;2(3):1-7.
27. Uddin MN, Farooq MM, Waqas M, Khan NU, Khan WA, Khan I, *et al* Antibiotic assays of *Salmonella* isolated from poultry chicken of various locations in districts Swat. *PAB*. 2018;7(1):78-84.
28. Van den Bogaard AE, London N, Driessen CAGG, Stobberingh EE. Antibiotic resistance of faecal *Escherichia* coli in poultry, poultry farmers and poultry slaughterers. J. of Antimicrobial chemotherapy. 2001;47(6):763-771.
29. Yadav MM, Roy A, Sharda R, Arya G. Detection of toxin genes and antibiogram pattern in *Escherichia coli* isolates from sheep meat on Indian market. Veterinarski Arhiv. 2007;77(6):485-494.
30. Yokoigawa K, Inoue K, Okubo Y, Kawai H. Primers for amplifying an alanine racemase gene fragment to detect *E. coli* strains in foods. J of Food Science. 1999;64(4):571-575.