

Research Article

Isolation, Identification and Antibiotics Susceptibility Test of *Escherichia coli* from Abattoir and Butchers in Bishoftu Town

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Abstract

E. coli with multiple antibiotics resistance is one of the public health issues concerned with the Food of animal origin safety. To control this pathogen; isolation and determining of antimicrobial resistance is a key tool. Therefore, the objective of the current study was to isolate, identify and detect the antimicrobial resistance of *E. coli* isolated from meat sample collected from selected slaughter house and Butchers in Bishoftu town central Ethiopia. Purposive sampling techniques were applied to collect 80 samples from which 47 Carcass swab. 12 material swabs and 21 carcasses from the selected abattoir (32) and Butchers (48). Conventional bacteriological laboratory culturing, on Eosin-Methylene blue to isolate general *E. coli* and on Sorbitol MacConkey agar to isolated pathogenic *E. coli* from general *E. coli* and biochemical test for *E. coli* identification and Disc diffusion antimicrobial susceptibility test were applied to isolate The *E. coli*. Out of 80 samples tested 18.75% of the samples were positive for the occurrence of the *E. coli*: The incidence of *E. coli* occurrence in two different meat value chain (Abattoir and Butcher) indicated the frequency of 25% and 14.5% respectively with no significant difference between them (P-value > 0.05). For antimicrobial sensitivity test out of eleven (11) antibiotics disk selected, two drugs, namely; Ampicillin and penicillin were 100% resistant to fifteen *E. coli* isolates; while fifteen isolates of *E. coli* were 100% susceptible to Gentamycin and sulphametazole. The antibiotic resistant test result in current study indicated that each *E. coli* isolate was resistant to at least two antibiotics. Therefore, Good hygienic and safety practice should be applied both at slaughter house and butchers, awareness creation among the society about the risk of transmission, prevention and controls of multidrug resistant *E. coli* should be given.

Keywords

Abattoir, Antimicrobial, Bishoftu, *E. coli*, Isolation

1. Introduction

Animal originated foods are usually associated with the high-risk of pathogenic microorganism agents, toxic component, and other transmissible contaminants [28]. Food-borne microorganisms are major pathogens affecting food safety and cause human illness worldwide [1]. *Escherichia coli* is the most considerable food-borne microorganism that causes

food-borne illness to consumers [26]. Enterohemorrhagic *Escherichia coli* O157: H7 is Major pathogenic microorganisms that frequently have been associated with foods of animal origin that may causes asymptomatic infection to severe diarrhea and/or hemolytic-uremic syndrome (HUS) [8]. Shiga toxin-producing *E. coli* is recognized as an important cause of

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food-borne disease in humans and causes large outbreaks worldwide [13, 22, 23]. *E. coli* O157: H7 is another species of *Escherichia coli* that cause of hemolytic-uremic syndrome (HUS) and hemorrhagic colitis with consequence of acute renal failure, hemolytic anemia, microangiopathic and thrombocytopenia to humans [10].

Escherichia coli (*E. coli*) is a gram-negative bacterium, facultatively anaerobic and a member of the Enterobacteriaceae family [25, 27]. It is commensal flora of warm-blooded animals gasro-intestinal and causing opportunistic infection (7; 9). Food contamination by pathogenic *E. coli* can occur at different the food value chain, abattoirs and processing, during distribution, butchers, preparation kitchen and meal tables [24]. *Escherichia coli* that can producing Shiga toxin infect the human when they consume contaminated meat, raw milk, and other animal products [11, 14].

People of all ages are susceptible to infection with STEC. However, the young and the elderly are more susceptible and are more likely to develop more serious symptoms [12]. Domestic and wild animals are sources of EHEC O157: H7 but the major animal carriers are healthy domesticated ruminants, primarily cattle, and to a lesser extent, sheep, and possibly goats. Fresh meat and raw milk are, nevertheless, considered common vehicles for *E. coli*, particularly for the EHEC (O157: H7) strain. Contamination of meat usually occurs during animal slaughter, because of poor slaughter practices, abattoir hygiene, and animal handling practices [21]. Detection of *E. coli* O157: H7 from food samples requires enrichment and isolation with selective and/or indicator media but lacks specificity to identify STEC [17, 20]. In Ethiopia, both food shortage and lack of appropriate food safety assurance systems are problems that have become obstacles to the country's economic development and public health safety [5]. Therefore, the main objective of the current study was to isolate antibiotics resistant *E. coli* from selected Slaughter house and Butchers in Bishoftu town Central Ethiopia.

2. Materials and Methods

2.1. Study Area

The study was conducted from March 2024 to May 2024 in and around Bishoftu town. Bishoftu is a town in central Ethiopia that Located in the East Shewa Zone of the Oromia Region, it sits at an elevation of 1,920 metres (6,300 ft). Bishoftu is approximately 47.9 kilometers (29.8 miles) southeast of Addis Ababa, along its Route 4 highway. The maximum temperature of an average day ranges between 17°C and 21°C and the average minimum temperature rarely drops below 2°C or rises above 32°C. This area receives an average annual precipitation of approximately 902mm (35.5 inches). The livestock resources of the area consist of cattle (bovine), sheep and goats, poultry, horse and donkeys, beekeeping, fish farming and small ruminants.

2.2. Study Design and Study Population

A cross sectional study was used to isolate, identify and detect antimicrobial sensitivity of *E. coli* in abattoir and butchers in Bishoftu town, East Shewa zone, Oromia. According to this study purposive sampling techniques was applied to collect 80 samples collected from the selected abattoir and butchers, conventional bacteriological laboratory culturing and biochemical test were conducted to isolate the *E. coli*, finally antimicrobial susceptibility test was conducted for the determination of resistance pattern of each *E. coli* isolates.

2.3. Sampling Techniques and Sample Collections

According to this study 32 samples were collected from 1 abattoir, while 48 samples were collected from 4 butchers in Bishoftu town. From the selected abattoir and butchers, 47 carcass swab and 12 surface contact material swabs were collected by using sterile cotton tipped swab and transferred in to 10ml test tube filled with 9 ml of buffered peptone water. The Remaining 21 samples of study were meat sample directly collected through direct collection of 25 g weighing tissue from butcher and abattoirs of whole cuts of raw meat sample was collected from abattoir and butchers following aseptic techniques. Then meat samples were put in a sterile plastic bag. Finally, each sample were labeled with permanent markers and handled in ice box and transported to AAU-CVMA Microbiology Laboratory. Then, after arrival the swab samples were directly incubated at 37°C for 24 hours for bacterial enrichment.

2.4. Sample Preparation and Enrichment

Each carcass swabs were homogenized with vortex mixer and 25 g of raw whole cuts of meat sample collected from each butcher and abattoirs were taken out from the universal bottle, chopped aseptically and the meat was placed with 225 ml of buffered peptone water; from the previous universal bottle; in a plastic bag and homogenized using a homogenizer and at high speed for 2 minutes and incubated at 37°C for 24 hrs. The resulting suspension was used for isolation of *E. coli*.

2.5. Isolation and Identification of Bacteria of *E. coli*

After 24 hours of sample preparation and enrichment, a sterile loop was dip into already enriched samples and streaked onto MacConkey agar plates as a differential media for identification of *E. coli*. Then, the plates were inverted and incubated at 37°C for 24 hours. After incubation period, the plates were examined for typical and atypical colonies. Typical colonies of *E. coli* grown on MacConkey agar are dry, medium in size, pink in color and appeared singular or in groups. Atypical colonies were small red colonies in singular or group form. Then typical colonies of *E. coli* were: Sub-cultured on

Eosin Methylene Blue (EMB) Agar and incubated at 37°C for 24hrs. Morphologically typical colonies was producing metallic sheen and under the same conditions to get pure colonies of *E. coli*. After the next 24 hrs. of incubation, well-isolated colony was selected and sub-cultured on Sorbitol MacConkey agar for appreciation of whitish colony, of *E. coli*. Again colony with white color was subculture onto Nutrient agar (NA) so as to be used for biochemical confirmation Stage 3: Biochemical confirmation of *E. coli*: Tests such as Gram staining and biochemical reactions like Oxidase, Catalase, Indole, Methyl red, Voges Proskauer (VP), Citrate (IMViC) and Triple sugar iron (TSI) tests were done on well-isolated colony from nutrient agar plates to confirm the presence of *E. coli* in the test samples. Colonies producing, positive for tryptophan utilization (indole test) (red ring), positive for Methyl red, negative for citrate utilization (green slant), negative for Voges-Proskauer (VP) and positive for Triple sugar iron (TSI) (yellow slant, butt and gas formation) test were *E. coli* positive.

2.6. Antimicrobial Susceptibility Testing

The isolates' antimicrobial susceptibility test was carried out using the Kirby Bauer disk diffusion method following the Clinical and Laboratory Standards Institute of the United States of America (16) and the Kirby Bauer Disk Diffusion Susceptibility Test Protocol on Muller Hinton agar medium. A sterile loop was used to transfer a loop full of well-grown colonies on nutrient agar from each biochemically confirmed isolate into sterile tubes containing 5ml of normal saline solution. The inoculated colony was mixed with saline solution by vortex until a smooth suspension was formed. Saline solution (if the suspension will be more turbid) or colonies (if the suspension was less turbid) was added until it met the 0.5 McFarland turbidity standards. Then the colony was swabbed uniformly over the entire surface of the Muller Hilton Agar plate using a sterile cotton swab dipped in the suspension and allowed to dry the plates by keeping at room temperature for 3 minutes in a bio safety cabinet [16].

Eleven antimicrobial disks with the known concentration of antimicrobial was placed on the Muller Hinton Agar plate and the plates was incubated for 24 hrs at 37°C. Each isolate was tested for a series of eleven antimicrobials: Tetracycline (TE) (10mg), Oxytetracycline (OT) (30mg), Penicillin (PEN) (10mg), Cotrimoxazole (COT) (25mg), Ciprofloxacin (CIP) (5mg), Gentamycin (GEN) (10mg), Streptomycin(S) (25mg),

Sulphamethoxazole (RL) (100mg), Ampicillin (AMP) (10mg), Azithromycin (AZM) (15mg) and Nalidixic acid (NA) (30mg). Using a caliber, the diameters of the clear zones of inhibition produced by diffused antimicrobial on lawn inoculated bacterial colonies was measured to the nearest mm. According to the published interpretive chart, all eleven zones of inhibition against eleven antimicrobial agents for each isolate was recorded, compared to standards, and classified as resistant, intermediate, or susceptible [16].

2.7. Data Management and Statistical Analysis

The collected data were entered and saved in MS-excel (MS Excel, 2007) and analyzed using statistical software. Before subjected to statistical analysis, the data was screened for errors and properly coded. P-value were employed to assess the presence of association. In all cases $p < 0.05$ were considered for statistically significant difference whereas p -value > 0.05 considered non-significant.

3. Results

3.1. Prevalence of *E. coli*

Out of eighty (80) samples purposively collected and examined under conventional culturing and Biochemical test for colony isolation and characterization; 18.75% (15/80) of the samples were positive for the occurrence of the *E. coli*. In study different risk factors including: sample sources, sample type and point of meat value chain from which samples were collected was included. According to Table 1, different sample sources of meat and swab, there was a different in frequency of *E. coli* occurrence in meat and slaughter material, despite there was no statistically significant difference among each sample sources (P -value > 0.05). The comparison between sample types indicated that the frequency of *E. coli* in carcass was higher than that of swab samples as its incidence were (9/21) 42.8% and (6/59) 10.1% respectively with its statistical differences (P -value < 0.05). Also, the incidence of *E. coli* occurrence in two different meat value chain (Abattoir and Butcher) indicated the frequency of (12/32) 25% and (3/48) 14.5% respectively with no statistical difference at two of them ($P = 0.09$).

Table 1. Prevalence and risk factors associated with *E. coli*.

Variable		Total sample examined	Positive	Incidence (%)	χ^2	P-value
Sample sources	Meat	68	12	17.6%	1.2854	0.2569
	Slaughter materials	12	3	25%		

Variable		Total sample examined	Positive	Incidence (%)	χ^2	P-value
Sample Type	Carcass	21	9	42.8%	20.213	0.000006
	Swab	59	6	10.1%		
Point of sample collection	Abattoir	32	8	25%	2.7911	0.09479
	Butcher	48	7	14.5%		

3.2. Antibiotics Resistant Pattern of *E. coli*

Table 2. Antimicrobial Resistance Pattern.

No. of Antibiotic	Resistant pattern and number of Isolate	Total numbers of resistant isolates (%)
2	AMP, P (1)	1(6.7%)
3	AMP, P, OT (1)	1(6.7%)
	TE, AMP, P, RL (1)	
4	NA, CIP, AMP, P (1)	4(26.7%)
	TE, CIP, AMP, P (1)	
	COT, NA, AMP, P (1)	
5	TE, AMP, P, RLOT (1)	2(13.3%)
	COT, AMP, P, RLOT (1)	
6	NA, TE, AMP, P, RLOT (1)	1(6.7%)
7	COT, TE, AMP, P, AZT, RLOT (1)	3(20%)
	NA, TE, CIP, AMP, P, RLOT (1)	
	COT, NA, TE, AMP, P, RLOT (1)	
8	COT, NA, TE, CIP, AMP, P, RLOT (3)	1(6.7%)

Out of eleven (11) antibiotics disk selected for antibiotics susceptibility test, according to Table 2, two drugs, Ampicillin and penicillin were 100% resistant to fifteen *E. coli* isolates; while fifteen of *E. coli* were 100% susceptible to Gentamycin and sulphametazole. Azithromycin was susceptible to thirteen isolates, and intermediate to one isolate and resistant to one isolate of *E. coli*. The remaining antibiotics disks were intermediate to some isolates and resistant to the other isolates of *E. coli*. This resistance was developed as the wide use of antimicrobials for either therapeutic or prophylactic purposes and growth promotion in livestock. The multi-drug resistant features of salmonella isolates are shown in Table 2. of all 15 *E. coli* isolates subjected to eleven (11) antibiotic susceptibility, all isolates were resistant to two (2) or more antimicrobials. Multi-drug resistant salmonella isolates showed 13 different resistant patterns for eleven antibiotic agents tested. Out of 26 multidrug resistant isolates, 1(6.7%) isolates were exhibited resistance to two antibiotics. Whereas, 1(6.7%) of the isolates

were resistant to three. Antibiogram profile also indicated that 8 (30.7%) of salmonella isolates were resistance to five antibiotics, seven isolates were resistant to seven antibiotics, two isolates were resistant to seven antibiotic s used in study. Only one isolate of salmonella was resistant to eight antibiotics (Table 2).

4. Discussion, Conclusion and Recommendations

Gastroenteritis due to foodborne disease is one of the most common illnesses iEthiopia, due to the. Consumption of raw beef is commonly practiced in Ethiopia. Generally, illegal slaughtering of animals in open fields, unhygienic slaughter practices in the abattoirs, and wide-spread tradition of consumption of raw meat (Kitfo dulet and Kurt) are potential risk factors in the country. Given that ruminants are natural reservoirs of Shiga toxin-producing Escherichia coli strains, higher

prevalence is common in areas where fecal contamination is continuous, namely, farms, transportation trucks used to deliver animals to slaughtering houses, and slaughtering halls [3]. Hence, the current study was conducted to determine the prevalence of multi-drug resistance *E. coli* in Bishoftu, central Ethiopia.

The comparison between sample types indicated that the frequency of *E. coli* in carcass was higher than that of swab samples as its incidence were (9/21) 42.8% and (6/59) 10.1% respectively. From the total of eighty (80) samples examined for the presence of *E. coli*, 18.75% (15/80) of the samples were positive for the occurrence of the *E. coli* in study samples. This result was greater than the prevalence of 16 (3.9%) *E. coli* O157: H7 contaminated reported by Ajuwon *et al.*, (2), also it was greater than overall estimated prevalence of 8.87% and 6.45% at abattoirs reported by (Ayisheshim *et al.*, (4) from Awi Zone, Northwest Ethiopia, despite the prevalence at butcher (11.29%) was relatively similar. the incidence of *E. coli* occurrence in two different meat value chain (Abattoir and Butcher) indicated the frequency of (12/32) 25% and (3/48) 14.5% respectively with no statistical difference at two of them ($P = 0.09$). relatively the overall prevalence in current study was similar with, 23.4% prevalence from Abattoir and Retailer Shops in Ambo Town, Ethiopia by [24].

According to the current study *E. coli* was recovered from beef, carcass swab and slaughter materials. The recovery of pathogen from the slaughter materials indicated that there were the risks of contamination of many beefs from single infected or contaminated beef during carcass processing in abattoirs. The tools and equipment used in the slaughterhouse such as knives, machines, non-potable water, workers hands, hooks, tables, and carriages contribute to the transmission of *E. coli* O157: H7 to blocks and minced meats [18]. Hence the *E. coli* O157: H7 live in the intestinal tract of livestock, it may be contaminated the carcasses during slaughter and evisceration [2]. However, the prevalence at abattoir were great difference, the prevalence at butcher was greed with retailer prevalence reported by Bekele *et al.*, [6] who reported 5.7% (11/192) from abattoir and 14.6% from retailer. Also, the contamination may occur namely, farms, transportation trucks used to deliver animals to slaughtering houses, and slaughtering halls [3].

The current study reveal that there was the higher prevalence of *E. coli* at slaughterhouses this indicated unhygienic processing facilities (19). Low prevalence of good hygienic practices resulted from attitudes, cleanness and disinfection of waste containers, regular supervision by health offices, health and safety training and Removal of personal items during meat processing May associated with the prevalence of *E. coli* reported in the current study [28]. According to the study conducted in n Mekelle Ethiopia by Gugsu *et al.*, [15] resistance gene developed by *E. coli* play an important role against routinely used antibiotics the prevalence reported in this study was indicated that there was contamination at two meat value

chain without no statistical difference. so that from this prevalence indication is easy to estimated consumer can be infected with *E. coli* because of the consumption of contaminated foods, mainly animal products contaminated with pathogens or their toxin [1].

Out of eleven (11) antibiotics disk selected for antibiotics susceptibility test, according to Table 2, two drugs, Ampicillin and penicillin were 100% resistant to fifteen *E. coli* isolates. The resistance to Ampicillin and tetracycline was greed with the resistance reported by Duc *et al.*, 2024 that indicated ampicillin (91.67%) and tetracycline (91.67%) resistance, the resistance to the antibiotics discs in this study agreed with the study of the [19] that reported the resistance frequency to ampicillin, tetracycline, gentamicin, and chloramphenicol were higher when it compared with the remaining antimicrobials. This resistance was developed as the wide use of antimicrobials as therapeutic or prophylactic purposes and growth promotion in livestock [4]. Additionally self-prescription and misuse antibiotics some practice behind the emergence of antibacterial (21).

In this study, the presence of *E. coli* in abattoirs and butchers shows moderately higher level. This indicates a significant level of contamination in the meat production environment. The *E. coli* isolates exhibited high resistance to penicillin and ampicillin, while showing medium resistance to other antibiotics tested. Additionally, some isolates were found to be multi-drug-resistant (MDR), demonstrating resistance to multiple classes of antibiotics. These findings raise concerns about the effectiveness of current hygiene practices and the use of antibiotics in meat production, as well as the potential health risks posed to consumers. The presence of MDR *E. coli* emphasizes the need for improved antibiotic stewardship and the implementation of stricter hygiene protocols in both abattoirs and butcher shops. Overall, the study underscores the importance of addressing contamination and resistance issues to protect public health. From the above Conclusion the following recommendation was forwarded: Good hygienic and safety practice should be applied both at Slaughter house and Butchers. Training about the risk of transmission, prevention and controls of multidrug resistant *E. coli* should be given for abattoir worker, Butchers and consumers, establish regular monitoring and surveillance programs to detect and track the prevalence of *E. coli* and other pathogens in meat production and retail environments and Further research should be conducted in the future to identify *E. coli* in the strain level.

Abbreviations

BPW	Buffered Paptone Water
EHEC	Enterohemorrhagic Escherichia Coli
EMB	Eosin Methylene Blue
STEC	Shiga Toxin Producing Escherichia Coli
TSI	Triple Sugar Iron Agar

Author Contributions

Ephrem Shimelis: Conceptualization, Formal Analysis, Writing – original draft

Muluken Tekle: Supervision, Writing – review & editing

Serkaddis Alem: Data curation, Investigation, Methodology

Conflicts of Interest

The authors declare no conflicts of interest.

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