Isolation and identification of *Escherichia coli* from raw meat at selected export abattoirs and retail houses in Bishoftu town, Ethiopia

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ABSTRACT

Raw meat is one of the commonly consumed traditional diets in Ethiopia. However, unhygienic processing and distribution practices are risky for contamination of meat leading to human infection. Consumption of meat contaminated by *Escherichia coli* (*E. coli*) causes a serious illness and even death to affected individuals. This cross sectional study was conducted from July, 2021 to September, 2021 at Abysinia export abattoir, Elfora Export Abattoir and 18 retail houses in Bishoftu town to isolate and identify *E. coli* from beef and shoot meat. The samples were transported to microbiology laboratory and isolation and identification of an organism was performed based on techniques recommended by international organization for standardization (ISO-16654: 2001) through morphological, cultural and biochemical characteristics. Accordingly, A total of 93 samples including beef (n= 38), sheep meat (n=29) and goat meat (n= 26) were collected from those export abattoirs and retail houses. Those samples were pre enriched in Tryptone soy broth followed by selective enrichments. *E. coli* were isolated and identified by colony characteristics on selective agar like MacConkey (MAC) agar, Eosine-methylene blue (EMB) agar, Gram staining and biochemical tests (Indole, Methyl red, Voges-Proskauer test, Triple Sugar Iron, Catalase, and Simon citrate agar test). The overall prevalence of *E. coli* in all food samples was 33(35.5%). A total of 12 (41.4%) sheep meat, 3(11.5%) goat meat and 18 (47.7%) beef samples were *E. coli* positive in which this difference is statistically significant (p.value= 0.01). The Abyssinia export abattoir was found to be the highest contaminated site 15 (42.86%) followed by ELFORA export abattoir 13 (32.50%) and retail houses 5(27.78%), respectively. However, there was no significant difference (P>0.05) between the sample collection area. The study evidenced a considerable presence of *Escherichia coli* in ruminant’s meat probably due to the poor sanitary conditions during production and processing of these foods. Therefore, improving the knowledge and practice of abattoir and retail house workers about safe meat handling and distribution may have great implications in the prevention and control of foodborne infections.

Key words: Abattoir, Bishoftu, *Escherichia coli*, identification, meat, retail houses, isolation.

ABBREVIATIONS

**EMB**: Eosin Methylene Blue; **ISO**: International Organization for Standardization; **MR**: Methyl red; **TSB**: Tryptone Soya Broth; **TSI**: Triple sugar iron; **VP**: Voges–Proskauer.

INTRODUCTION

Foods of animal origin like shoot meat and beef are rich in proteins which are very essential to body growth and development. However, foods of animal origin also act as a vehicle and medium to transmit various microorganism causing health hazards, disease and death. Food borne diseases are a growing public health problem all over the world which cause an estimated 48 million illnesses and 3,000 deaths each year in the United States (Scallan et al,
In developed countries, up to 30% of the population suffers from food borne diseases each year, whereas in developing countries up to 2 million deaths are estimated per year (WHO, 2007a, b). In Ethiopia also, the occurrence of E. coli in foods of animal origin is arguably high due to many reasons like unhygienic slaughtering practices in the abattoirs, illegal slaughtering of animals in open fields, poor meat transport, and display conditions at butcher shops (Dulo et al., 2015). E. coli is one of the major food borne bacterial pathogen. Majority of the E. coli are non-pathogenic but few of them are highly pathogenic causing watery and bloody diarrhoea e.g., E. coli 0157:H7 which is associated with life threatening disease such as hemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (Mohamed et al., 2014). E. coli is characterized as a gram-negative, facultatively anaerobic, rod-shaped, and highly motile bacteria that belong to the family Enterobacteriaceae, and a normal inhabitant of the intestines of animals and humans (Virpari et al., 2013; Asmelash, 2015) but its recovery from food may be of a public health concern due to the possible presence of enteropathogenic and/or toxigenic strains which lead to a wide variety of enteric and extraintestinal diseases in animals (Asmelash, 2015). There are five virulence groups of E. coli, including: enteraggregative, enterohemorrhagic, enteroinvasive, enteropathogenic, and enterotoxigenic (Assefa and Bihon, 2018).

Meat is one of the most nutritious and favorite animal-source foods. Due to its high water content (0.99 water activity) and being rich in proteins, minerals, and other nutrients which are suitable for microbial growth, meat is a highly perishable food that can cause infection in humans and also can lead to economic loss due to spoilage (Thanigaivel and Anandhan, 2015). Meat can be contaminated by E. coli during animal slaughter due to unhygienic slaughter practices, through airborne, rodents, insects, and other animals (Laury et al., 2009). Consumption of contaminated and/or uncooked meat poses the risks of acquiring foodborne E. coli strains causing a serious public health concern (Frye and Jackson, 2013). Escherichia coli can be differentiated from other members of the Enterobacteriaceae based on several sugar-fermentation and other biochemical tests. Classically important groups of tests used for this purpose are known by the acronym IMViC. These tested for the ability to produce: indole from tryptophan (I), sufficient acid to reduce the medium pH below 4.4, the breakpoint of the indicator methyl red (M), acetoin (acetyl methyl carbinol) (V), and the ability to utilize citrate (C) (Adams and Moss, 2008). Despite that E. coli can be identified with a variety of biochemical reactions, the indole test remains the most useful method to differentiate E. coli from other members of the Enterobacteriaceae (Xia, 2010). A handful of studies have been conducted in Ethiopia that reports the occurrence level of E. coli in foods of animal origin mostly in meat in recent years (Atnafie et al., 2017; Dulo et al., 2015; Mengistu et al., 2017; Taye, 2013). Most of the studies were conducted in central Ethiopia due to extensive animal farming practices in those areas (Assefa and Bihon, 2018). Contamination of meat with foodborne pathogens is a major public health issue. Hence, studies on the prevalence of bacterial pathogens on meats are important to estimate the level of contamination of meat. Therefore, the main objectives of the present study were to determine and compare the isolation of E. coli in export abattoirs and retail houses and to determine and compare the isolation of E. coli in beef, sheep, and goat meats.

MATERIALS AND METHODS

Study area

The study was conducted in retail houses, Bishoftu ELFORA and Abysinia export abattoir in the town of Bishoftu which is located at 9°N latitude and 40°E longitude from July 2021 to September 2021. The geography of the area is marked by creator lakes and is located at about 45 km Southeast of Addis Ababa at an altitude of 1880 meters above sea level. It has a total human population of 95,000. The area receives an average annual rainfall of 800 mm and has an average maximum and minimum temperature of 30.7°C and 8.5°C, respectively, and the mean relative humidity is 61.3% (CSA, 2013).

Study design

A cross-sectional study design was conducted from July 2021 to September, 2021 in purposively selected export abattoirs and retail houses found in Bishoftu town, using microbiological procedure to isolate and identify E.coli from selected sites.

Study population and sample type

The study populations were ruminants (bovine, sheep and goat). Raw meat sample from those ruminants was collected from selected abattoir and retail house in Bishoftu town.

Sample collection

A total of 93 samples were collected; 15 from retail house, 40 from export abattoir of ELFORA and 35 from Abysinia. The sample size was fixed based on the representative samples taken from selected sites. Probability sampling (simple random) was used to select the population to be sampled. Samples were taken from two export abattoirs (ELFORA and Abysinnia) and eighteen retail houses.
Accordingly, 38 beef sample, 29 sheep meat and 26 goat meat were collected. First, animals were selected using a systematic random sampling technique from a list of animals that were brought to Bishoftu export abattoirs and retail houses. Meat samples were collected from different parts of the carcasses (abdomen [flank], thorax (lateral) and breast (lateral), brisket, and crutch) at abattoir house and whole cuts of raw meat at butcher shops as per the International Organization for Standardization (ISO 16654, 2001). A total of 25 g of meat was taken from the relevant places of the carcasses at the abattoir house and whole cuts of raw meat at butcher shops using sterile scalpels and forceps and put into a sterile, separately labeled plastic bag and each pooled meat sample was thoroughly homogenized. Meat samplings were made after the removal (evisceration) of the gastrointestinal tract and displayed for sale at butcher shops. After each sampling, separate scalpel and forceps were cleaned with pieces of gauze dipped in 70% ethanol to minimize cross-contaminations and immediately transported to the microbiology laboratory of the College of Veterinary Medicine and Agriculture in an icebox with ice packs and processed within 8 h.

Sample preparation and isolation procedure

Raw meat samples collected from abattoir and retailer shops were taken out of plastic bags using sterile thumb forceps. The E. coli isolates were identified by using standard bacteriological methods, comprising of colony structure determination, using different culture media and biochemical tests as previously indicated (Hitchins et al., 1998). From each chopped and mixed meat sample, 25 gm was transferred into a plastic bag (Seward, England), containing 225 ml of tryptone soya broth (TSB) (Himedia, India). The resulting homogenate was incubated at 37°C for 24 h. All pre-enriched meat samples were subsequently sub-cultured onto MacConkey agar and incubated at 37°C for 24 h. Five to ten suspected colonies of E. coli (pinkish color appearance) were sub-cultured onto separate Eosin Methylene Blue (EMB) agar. A loopful of a pure culture of presumptive colonies from each sample was streaked on nutrient agar (Himedia, India) and incubated for 24–36 h at 37°C for gram stain and confirmed by biochemical tests: using, production of indole (positive), methyl red (positive), Voges-Proskauer test (negative), Triple Sugar Iron (AG/A), Catalase (positive), and Simon citrate agar test (negative) were considered as E. coli (Abdissa et al., 2017).

Sample size determination

Purposive sampling technique was applied on all available abattoir and retail house in the study area. A total of 2 abattoirs and 18 retail house in Bishoftu were selected conveniently based on the availability of the samples. The sample size was calculated according to the formula given (Thrusfield, 2007) using 74.7% previous prevalence of clinical and subclinical mastitis (overall) in the same area (Zeryehun et al., 2013) 5% absolute precision and 95% confidence level.

\[ n = \frac{1.96^2 \times p \times (1-p)}{d^2} \]

Where \( n \) = required sample size, \( p \) = expected prevalence \( d \) = desired absolute precision and 1.96 is multiplier of 95% CI.

Data analysis

The prevalence of E. coli infection was quantified and compared among meat samples of different livestock species. The data were analyzed by using SPSS software version 20 and P-value was calculated using Chi-square to determine any significant correlation. A P-value less than 0.05 was considered statistically significant.

RESULTS

Prevalence of E. coli

In this study, out of the 93 meat samples collected a total of 33 (35.5 %) E. coli isolates were identified as seen in Table 1 and Figure 1. Of these positive cases, beef meat had the highest (47.37 %) whilst goat meat had the lowest prevalence (11.54 %) of E. coli. A significant difference in E. coli prevalence (P < 0.05) was observed among meat samples of different livestock species. The highest prevalence was observed in Abyssinia export abattoir (42.86%) whilst retail houses had the lowest prevalence (27.78 %) of E. coli (Table 2). There was no statistically significant difference (P>0.05) in the occurrence of E. coli between the two abattoirs and retail houses.

DISCUSSION

Food borne disease (FBD) has emerged as an important issue of growing public health and economic problem in many countries (Bouchrif, et al., 2009). Escherichia coli are among the most common food borne pathogens (Polpakdee and Angkititrakul, 2015) Escherichia coli is considered the most prevalent food borne pathogen that has gained increased attention worldwide in recent years. The present study was conducted to establish isolation and identification pattern of E. coli as well as associated risk factor on samples collected from abattoir and retail house in Bishoftu town. In the present study, from the total of 93 samples collected from abattoirs and retail houses 33 (35.5%) were positive for E.coli. The magnitude of E. coli prevalence in this study was similar to the previous studies.
Table 1: Prevalence of E. coli concerning sampling area and species of animals.

<table>
<thead>
<tr>
<th>Meat Sample</th>
<th>Total sample tested</th>
<th>No. of positives</th>
<th>Prevalence (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abyssinia</td>
<td>35</td>
<td>15</td>
<td>42.86</td>
<td></td>
</tr>
<tr>
<td>ELFORA</td>
<td>40</td>
<td>13</td>
<td>32.50</td>
<td>0.4834</td>
</tr>
<tr>
<td>Retail houses</td>
<td>18</td>
<td>5</td>
<td>27.78</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>93</td>
<td>33</td>
<td>35.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Sheep</td>
<td>29</td>
<td>12</td>
<td>41.38</td>
<td></td>
</tr>
<tr>
<td>Sample type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goat</td>
<td>26</td>
<td>3</td>
<td>11.54</td>
<td>0.01</td>
</tr>
<tr>
<td>Beef</td>
<td>38</td>
<td>18</td>
<td>47.37</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>93</td>
<td>33</td>
<td>35.5</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Prevalence of E. coli in retail houses, Abyssinia and ELFORA export abattoirs in meat sample of ruminants.

in different part of 35.21% Barua et al. (2007). However high rate than present finding was reported 40% in Ethiopia (Mekonnen et al., 2013), 37.86% in Bangladesh (Rahman et al., 2017), 62.26% in the Khon Kaen (Polpakdee and Angkititrakul, 2015). Low rate as compared with present finding was 22.2% by Haiiselassie et al. (2012), 26.6% by Haimanot et al. (2010) 20.3% by Bitew et al. (2010), 30.97% by Taye et al. (2013). These differences above (higher or lower prevalence) from present finding might be resulted from the difference in study design, isolation technique, different in sample type and amount and difference in geographical location, breeds of birds and types of chicken and difference in quality of works and the amount use antibiotic is the major cause.

With regard to sample source, 14 (50%) beef, 11(39.2%) sheep meat and 3(10.7%) goat meat of samples from the abattoir house and 4 (80%) beef, 1(20%) sheep meat and 0(0%) goat meat of samples from the retail house were positive for E. coli respectively. Despite the differences in proportion of positive samples, the presence of E. coli in abattoir and retail house sample were reported in many studies in Ethiopia (Bersisa et al., 2019; Dulo, 2014; Atnafie et al., 2017; Abdissa et al., 2017). In the current study the overall prevalence of E.coli was significantly higher in beef than sheep and goat meat. The overall variation observed among the reported prevalence could be emanated from difference in hygiene, breed, geographical origin. Observed variation in prevalence among studies could be attributed to difference in sampling and isolation procedures, fecal and skin contact to carcass, method of meat transportation to butcher house, method of rumen content removal, abattoir conditions, study design, season and treatment with antimicrobial substances during the process (disinfectants). A number of studies have confirmed that
the prevalence of *E. coli* O157:H7 varies among studies due to the above mentioned reasons (Chapman et al., 2009). According to the report by Bassam et al. (2012), the infective dose of the pathogen is < 10 cells for humans. Considering this very low infective dose of this pathogen, its detection in the retail houses and abattoir of this study poses public health risks.

**CONCLUSION AND RECOMMENDATIONS**

The present study evidenced a considerable presence of *E. coli* in ruminant meat slaughtered in abattoirs and retail houses in Bishoftu. The work revealed an overall *E. coli* prevalence of 35.48% in raw meat samples of beef, sheep, and goat origin. The occurrence of the bacteria in raw meat samples significantly differed concerning the different species of animals. However, the difference in prevalence regarding samples collection area was not significant. In general, the poor sanitary conditions during meat processing appear to highly contribute to carcass contamination by *E. coli*. All these findings suggest that the consumption of undercooked meat or food cross-contaminated with *E. coli* may pose a serious threat to consumer health. Therefore, based on the above conclusion the following recommendations are forwarded:

1. Coordinated actions are needed to reduce or eliminate the risks posed by this organism at various stages in the food chain.
2. Detailed inspections and monitoring of slaughter houses for proper meat hygiene; handling and sanitary practices should be a priority as an immediate intervention.
3. Improving knowledge and practice of abattoir and retail house workers about safe meat handling and distribution may have great implications in the prevention and control of food borne infections.
4. Meats should be properly cooked before consumption.

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**REFERENCES**


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