Isolation, Identification and Antimicrobial Susceptibility Test of E. Coli from Housed Dogsin Harar Town, Eastern Ethiopia

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Abstract:
Since having dogs live closely at home has become more common, epidemiological data are needed in order to prevent and control zoonotic pathogens such as *Escherichia coli* [1]. A cross-sectional study was conducted with the objectives of isolating, identifying, and assessing the risk factors for *E. coli*. From housed dogs found in Harar, a town in eastern Ethiopia. Households and dogs were selected purposefully based on the willingness of owners to provide data and allow dog sampling. A total of 384 dogs from 196 households were included in the study. From the study dogs, rectal samples and fresh feces swabs were taken aseptically using sterile cotton swabs, and samples were transported using a cold chain. Prior to sample collection, individual animal information and dog management practices were collected using an appropriate format. The swab samples were subjected to a bacteriologic culture of *E. coli* using the recommended guidelines. Further identification was done based on cellular response, morphology, and biochemical identification of isolates. Descriptive statistics and chi-square analysis were used to present the data using the SPSS statistical package. The overall prevalence of *E. coli* were 27.6% and 19.3% (95% CI = 15.4–23.6) at the household and dog level, respectively. *E. coli* was found in all the studied *Kebles* with a significant difference (2 = 16.086; P = 0.003). The prevalence was significantly higher (P = 0.028) in clinically ill dogs (36.0%) than in apparently healthy ones (18.1%; 95% CI = 14.3–22.5). Among the studied variables, breed of dog showed a significant association (2 = 5.489; P = 0.019) with *E. coli* isolation, in that it was higher in local breeds (21.9%) compared to exotic breeds (10.9%). Although the prevalence was not significantly different among other variables, a relatively higher prevalence was observed in young dogs (6 months), females, and thin animals than the other categories. Meanwhile, it was higher in dogs fed on offal and fed uncooked preparations. Generally, *E. coli* is frequently isolated in apparently healthy and clinically ill dogs in the study area. Therefore, it is important to consider the exposure factor for better pet management so as to improve the livelihood of dogs and prevent the possible transmission of zoonotic *E. coli* to pet owners and those in close contact with the animals.

Keywords: Eastern Ethiopia, E. coli, Harar town, Housed dogs, Risk factors.

Introduction:

As humans have been living alongside dogs for tens of thousands of years.
Their function in our life has evolved from protector to farm worker to, most recently, pet to family member. Around 6 million dogs are thought to exist in Canada, with at least 30 to 40 percent of homes owning one or more of them [2].

Modern dogs are not just kept as family pets but increasingly as therapy and service dogs, which exposes new and potentially vulnerab le populations of people to zoonotic infectio ns[3].

The majority of individuals have intimate ties with their pet dogs, which necessitates research into the potential zoonotic dangers connected to dog interaction, as does the rising exposure of vulnerable populations to dogs [4].

Pleomorphic, Gram-negative, nonspore-forming rods, Escherichia coli is a member of the Enterobacteriaceae family. Escherichia coli are a normal component of the intestinal microflora, but when local or systemic immunity is compromised and there are bacterial virulence factors, they can also be linked to gastroenteritis [5]. Diarrheagenic *E. coli* has been divided into a number of distinct pathotypes, and each pathotype is characterized by a unique set of virulence factors that were acquired through horizontal gene transfer and work together to determine the clinical, pathologic, and epidemiologic characteristics of the disease they cause[6]. The seven pathotypes include adherent-invasive *E. coli* (AIEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic E. coli (ETEC), enterohemorrhagic E. coli (EHEC), necro toxigenic *E. coli* (NTEC), and enter invasive E. coli (EIEC). 112–114 Major food-borne pathogens such as entero-hemorrhagic *E. coli* (EHEC) cause serious infections ranging from moderate diarrhea to hemorrhagic colitis and life-threatening consequences. The primary virulence factor of EHEC is shika toxins (Stxs) [7]

Many strains have been found in both dogs with and without diarrhea, and it is unclear how many of these strains contribute to the development of diseases in canines and felines. Contrarily, there is evidence that certain dog breeds, including the Boxer, French bulldog, and Border Collie, are susceptible to AIEC strains. It is also known as STEC [8]. Escherichia coli O157:H7 is now one of the causes of diarrhea worldwide. However, a strain of *E. coli O157:H7* causes bloody diarrhea when it grows in the intestines. Dogs and puppies regarded as important reservoirs for *E. coli O157:H7*, which is one of the main causes of diarrhea and other diseases in human (Hasan *et al.*, 2016). Diarrheic and non-diarrheic dogs of all ages may serve as potential sources of multi-drug resistant STEC O157:H7 transmissible to humans [1]. Escherichia coli, a ubiquitous commensal bacterium, is considered a major potential source of antimicrobial resistance genes. Recently, the prevalence of resistance to at least one antimicrobial in E. coli isolates from healthy pet animals has been estimated to be between 10% and 30% from surveillance and scientific studies in Europe and Canada [9]. Because E. coli is easily and inexpensively recovered in most animals and is a reservoir of antimicrobial resistance genes, generic E. coli is frequently used as an indicator organism for AMR surveillance [10]. Unfortunately, in some cases, E. coli was found to be generally poor at predicting the AMR patterns of pathogenic bacteria, such as Salmonella, recovered from the same animal [11].

However, generic E. coli can still be used to monitor potential emerging resistance issues that might be transferred to pathogenic bacteria. Since having dogs and cats living closely at home has become more common in area, having epidemiological data and patterns of antimicrobial resistance are needed in order to prevent and control *E. coli* in pets. In line with petdog's health management and control of zoonotic enteric pathogen, an investigation on the significance of occurrence of pathogens found paramount contribution. Moreover, investigation include isolation and identification of this pathogens under multifactorial structured study which will have paramount in reducing or prevention enteric pathogen. The information is essential for control management for the disease affecting animal, humans and public health in the area.

In Ethiopia, despite an increasing trend of keeping dogs in urban areas, limited study is available on the occurrence of zoonotic bacterial pathogens in dogs, particularly E. coli. Beside this, there is a paucity of documented data on this disease in dogs in the study area. Therefore, there is a need for study on E. coli so as to understand the risks for the disease distribution and estimate the risks for human transmission. The general objective of this study was to assess the prevalence, identify the associated risk factors, and determine the antimicrobial susceptibility test of E. coli from a fecal sample of housed dogs in Harar, Eastern Ethiopia.

Materials And Methods:

Study Area:

Harar town, is located between 9.11-9.24'N latitude and 42.03-42.16'E longitude at the distance of about 526 km East of Addis Ababa. The altitude of the town is 1850m above sea level and its mean annual rainfall and humidity is 596 mm and 60.3%, respectively. The town has mean annual maximum and minimum temperatures of 25^0c and 10^0C, respectively. The total human population of the town was estimated at 125,000 with a growth rate of 2.6% (CSA, 2013). An updated data from Harari region livestock and fishery office show that the number population in region 285,000 and from this 111,150 (39%) and 173,850 (61%) are Rural areas (17 Keble), Urban areas (19 kebles) respectively (kebles is the smallest administrative unit), Cattle 52,000, Equine 11,250, Goats 57,000, Sheep 63,000, Poultry 72,000 and Hives of Bee (Apiary) 1,290 in Nine woreda of the regional state (HRLFO, 2019).

Study Dogs and Inclusion Criteria

The target populations for this study were house hold dogs. The study animals comprised different age, breed, sex, and body conditions. It was observed that in all the household's dogs were housed either tied or untied. In all the households, kennel cleaning was performed without any detergent. The water used for dogs were potable and used for human consumption. In all the households there was no practice of adding drugs to feed or water except for the reason of medication. All dogs included for the study were those who didn't took antimicrobial medication 4 weeks prior to sample collection.

Study design:

A cross- sectional study was conducted from November 2019 to March 2020 to isolate and identify of E. coli species from and rectal swabs of dogs.

Sample Size Determination and Animal Selection:

The Sample size was calculated using the formula described by Thursfield (2018). The considerations during the sample size determination were, 95% confidence interval, 5% precision, and an expected prevalence of 50%. The formula is described below as:

$$n = \frac{1.96^2 X\ \text{Pexp}(1 - \text{Pexp})}{d^2}$$

Where n= sample size

Pexp= expected prevalence

d = desired absolute precision

Accordingly, 384 dogs were included for fecal sample examination.

Since there was no recorded data on dog ownership in the town, households and animals' selection were based on house to house movement and inclusion of those which were voluntary to provide information and allow dog sampling. Thus, selection is generally based on accessibility and voluntariness.

Sample Collection and Transportation

Prior to sample collection individual information such as sex, age, breed, and body condition was recorded in data collection sheet (Annex I). Body condition score was done based on 5 scale (emaciated, thin, ideal (medium), fat, and obese) according to AAHA (American Animal Hospital Association).

Rectal swabs and fresh uncontaminated feces were collected using a sterile cotton swab after proper restraining of the dogs with the help of dog owners. The rectal swabs were immediately placed in a sterile test tube containing buffered peptone water. The collected samples were then placed in a box containing an icepack and transported to Veterinary Microbiology Laboratory, Hara maya University.

3.6. Questionnaire Survey on Dog Management

In the present study, data was collected on antimicrobial medication (for inclusion criteria), and gastrointestinal illness. In addition, household management related to feed types, feed treatment, dog house cleaning, and housing conditions were collected (Annex I).

3.7. Isolation and Identification of Escherichia coli

Isolation and identification were performed according to the guidelines. The media used for isolation were MacConkey [3] agar and Eosine Methylene Blue (EMB) agar. The media were prepared according to the manufacturer's instructions (Annex II).

3.7.1 Isolation

A loop full of the enriched sample (sample in buffered peptone) was spread on MC agar and incubated at 37^0C for 24 hrs. Five representative typical colonies (pink colonies) from MC agar were then transferred on to EMB agar and incubated at 370c for 24 hrs. The organisms showing characteristic colony morphology of *E. coli* was repeatedly sub-cultured onto EMB agar until the pure culture with homogenous colonies were obtained. Then isolated colonies showing metallic sheen on EMB agar were transferred into nutrient agar for further biochemical characterization and morphological examination.

Identification:

Younger colonies (≤24 hrs) of suspected *E. coli* were subjected for Gram's staining to observe cellular morphology and gram's reaction. Furthermore, suspected isolates were tested for catalase reaction, oxidation-fermentation oxidase, indole production, methyl red, Voges–Proskauer (VP) reaction, citrate utilization, and motility. Generally, *E. coli* was considered if gram negative, motile, catalase positive, fermentative, oxidase negative, indole positive, MR positive, VP negative, citrate negative.

Data Management and Analysis:

The collected data were entered and stored in Microsoft excel spread sheet. The data was thoroughly screened for errors and properly coded before subjected to statistical analysis. Before analysis, the age of dog was classified in to three group young (< 6 months), mature (6 months to 2 years), and old (> 2 years). Feeding was categorized as uncooked and mixed. In the mixed category, the dog owner occasionally used to cook dog feeds. Then the data from Microsoft excel sheet was processed and analyzed by using statistical software program (SPSS) version 20. Descriptive statistics such as frequency and percentage were used to present the prevalence of *E. coli*. Moreover, Chi-square was employed to establish the association between *E. coli* and risk factors. In all cases the difference between parameters were tested for significance at probability level of less than 0.05 (P ≤ 0.05)

Results:

Household Prevalence of E. coli

The study revealed that out of the 196households examined *E. coli* was detected in 54 (27.6%). The prevalence was higher in *Keble* 10 (75%) as compared with the other *Kebles*. The prevalence was higher in households who fed their dog mainly offal's and had no practice of feeding cooked feed than the counter categories. Chi-square analysis revealed that there were statistically significant differences (p<0.05) in the prevalence of *E. coli* among studied Kebles (Table 1).

Table **1.** Household level prevalence of *E. coli* in *Kebles* of Harar town

Variables		**Total household examined**	**No. of household positive**	**Percentage**	***X^2 (P-value)***
Kebles	15	72	11	15.3	27.8 (<0.001)
	16	62	12	19.4	
	13	23	10	43.5	
	10	12	9	75.0	
	18	27	12	44.4	
	Sub total	**196**	**54**	**27.6**	
Feeding					
	Left-over	137	38	27.7	1.6 (0.448)
	Offal's	12	5	41.7	
	Mixed	47	11	23.4	
	Sub total	**196**	**54**	**27.6**	
Feed treatment					
	Uncooked	19	6	31.6	0.171 (0.679)
	Mixed	177	48	27.1	
	Sub total	196	54	**27.6**	

Dog Level Prevalence of E. coli and Risk factors:

Of the total 384 examined dogs, 74 (19.3%) were positive for *E. coli*. The prevalence was higher in *Keble* 10 (36.7%) as compared with the other Kebles. Chi-square analysis revealed that there were statistically significant differences (p<0.05) in the prevalence of *E. coli* among Kebles (Table 2).

Table2: Animal level prevalence of *E. coli* across the studied *Kebles* of Harar town

Kebles	Total No. of dogs examined	Number of dogs positive for *E. coli*	**Percentage**
15	101	12	11.9
16	137	20	14.6
13	61	18	29.5
10	30	11	36.7
18	55	13	23.6
Total	**384**	**74**	**19.3 (95% CI=15.4 - 23.6)**
CI= Confidence interval; Pearson Chi-Square16.086; P-value=0.003			

The study revealed that the prevalence of *E.coli* was higher in dogs of local breed (21.9%) as compared with the exotic breed (10.9%). Moreover, the prevalence was higher in young dogs (21.2%) as compared with other categories of age. The finding also showed that, the prevalence of *E. coli* was higher in dogs with GIT illness (36%) than apparently healthy. Chi-square analysis revealed that there were statistically significant differences (p<0.05) in the prevalence of *E. coli* among categories of breed and clinical condition (Table 3).

With regard to dog management related, the study showed that high prevalence of *E. coli* was observed in dogs fed on offal's (25) than mixed (19), and left over (18.8). Meanwhile, higher prevalence was observed in dogs fed with uncooked feed (25.8%) than mixed one (18.7%).

Table 3. Prevalence of *E. coli* and associated risk factors

Variables		**No. of animals examined**	**No. of animals with *E. coli* (%)**	**CI (95%)**	**χ2 analysis**	
					χ2 value	**p-value**
Sex						
	Male	260	48 (18.5)	13.9 - 23.7	0.339	0.560
	Female	124	26 (21.0)	14.2 - 29.2		
Age						
	Young	99	21 (**21.2**)	13.6 - 30.6	2.638	0.267
	Mature	66	8 (12.1)	5.4 - 22.5		
	Old	219	45 (20.5)	15.4 - 26.5		
Breed						
	Local	292	64 (**21.9**)	17.3 - 27.1	5.489	0.019
	Exotic	92	10 (10.9)	05.3 - 19.1		
Body condition score						

	Thin	259	60 (23.2)	18.2- 28.8	0.933	0.334
	Medium	89	14 (15.7)	8.9 - 24.9		
Feeding						
	Left-over	260	49 (18.8)	14.3 - 24.1	.541	0.763
	Offal's	24	6 (25)	09.8 - 46.7		
	Mixed	100	19 (19)	11.8 - 28.1		
Feed treatment						
	Uncooked	31	8 (25.8)	11.9 - 44.6	0.926	0.336
	Mixed	353	66 (18.7)	14.8-23.2		
Illness						
	No	359	65(18.1)	14.3 - 22.5	4.811	0.028
	Yes	25	9(**36.0**)	17.9 - 57.5		

AMR profile is outlined in Table 4 In the cases of E. coli, the highest resistance was found toward ceftriaxone (83.8%) and penicillin (79.7%), followed by sulfamethoxazole/trimethoprim (62.1%), ampicillin (54%), oxytetracycline (54%) and Ciprofloxacin (52.7). Only 16.2% of the isolates showed resistance to gentamycin

Table 4. In-vitro antimicrobial susceptibility of the isolated *E.coli* (n=74).

Antimicrobials	Abbreviation	Susceptible No. (%)	Intermediate No. (%)	Resistance No. (%)
Sulphamethoxazole	SUP	19(25.7)	9(12.2)	46(62.1)
Oxytetracyline	OTC	23(31.1)	11(14.9)	40(54.0)
Peniccillin G	PG	13(17.6)	2(2.7)	59(79.7)
Ceftriaxone;	CRO	9(12.2)	3(4.0)	62(83.8)
Erythromycin	ERY	36(48.6)	13(17.6)	25(33.8)
Gentamycin	GEN	59(79.8)	3(4.0)	12(16.2)
Ampicillin	AMP	23(31.1)	11(14.9)	40(54.0)
Cefoxin	CEF	27(36.5)	10(13.5)	37(50.0)
Ciprofloxacin;	CIP	24(32.4)	11(14.9)	39(52.7)

Discussion:

Dogs and cats, called companion animals, have a close relationship with humans. For that reason, a companion animal carrying EHEC may become a human health threat [12]. Apparently healthy dogs can harbor *E. coli* and might thereby serve as a potential source of human infection with implications for public health.

The current study showed that an overall percentage of 19.3% (95% CI: 15.4–23.6) of dogs in Harar, Eastern Ethiopia, harbor *E. coli* in their gastrointestinal tract. Among this group, 18.1% (95% CI = 14.3–22.5) carry *E. coli asymptomatically*. The current finding is higher than those reported by [13]from Japan (0.16%); [14]from Argentina (1.1%); and [2] (3.2 to 12.3%) in apparently healthy dogs. However, the results of the current study are lower than those reported by [15]from Nigeria, who showed that the percentage of *E. coli* in apparently healthy dogs was 26.9%. The prevalence of *E. coli* might have been higher if more than one rectal swab culture was performed on each dog. The limitations of single rectal swab cultures for isolation of *E. coli*, due to intermittent shedding, are well documented. Dogs with experimentally-induced latent infection shed the agent irregularly for the subsequent 3-4 weeks. In rare cases this shedding continues for up to 100 days. Since the agent is being shed at intervals, sampling times are very important when searching the carrier status of the dogs and in the present study we can only conclude that the dogs were positive or negative.

In the present study, it was found that *E. coli* prevalence was significantly varied across the studied Kebles at the household (*Chi-Square = 27.8*; p 0.001) and animal level (Chi-Square = 16.086; p = 0.003). The variation could be due to the dog management practices shared among neighbors, in that households in the same neighborhood have a higher probability of having similar management practices compared to households in other neighborhoods. This in turn might lead to similar disease patterns in animals managed in a particular area or place.

The high prevalence of *E. coli* in diseased dogs (36.0%) is higher than in healthy dogs (18.1%), indicating *E. coli* is one of the main enter pathogens most commonly identified in dogs and can be the direct cause of the diarrhea recorded, as previously mentioned by Brook (2006) for both acute and chronic diarrhea.

In the present study, bacteriological examination of samples revealed that the proportion of *E. coli* in dogs based on age category was 21.2%, 12.1%, and 20.5% in young, mature, and old dogs, respectively. Similarly, [16]reported higher percentages of *E. coli* and other bacteria in younger animals, indicating young animals have greater susceptibility for colonization by the pathogen.

The present study showed that *E. coli* was relatively higher in females. This may be due to decrease in estrogen levels and increasing progesterone levels during each ovulation, which leading to decline in immune status [17]. The high prevalence of *E.coli* that are typically reported in dogs fed on offal's compared to others may be traceable to the high-protein raw meat that favors for *E.coli* multiplication. Previous reports indicated that identical enter types have been found in the feces of dogs consuming the food, confirming that the diet is the likely source of *E.coli*. In some instances, the dogs may not be colonized by *E. coli* and may just be passive carriers in which foodborne *E.coli* is transiently passing through the intestines [18].

Antimicrobial resistance in bacteria is a phenomenon that has been in constant evolution since the introduction of antimicrobial drugs. Several factors are known to promote bacterial resistance including failure of a treatment regimen, prophylactic use of antimicrobials, and the use of antimicrobials as growth promoters as well as using antimicrobials commonly used in humans' practice[3]Antimicrobial resistance has been suggested as one important therapeutic problem in veterinary and human medicine [19]In this study, antimicrobial resistance of E. coli isolated from apparently healthy dogs was investigated against 9 antimicrobial drugs using the disk diffusion method. Overall, moderately low antimicrobial resistance was found (16.2%). Antibiotic sensitivity patterns might explore the possible MDR bacteria in dogs, and due to living in close contact with dogs, it may cause problems in humans if they are infected with MDR bacteria from dogs. The findings of the study reveal the common bacterial pathogens circulating in dogs, and also show their extended spectrum of resistance to several antibiotics that are commonly used in therapeutic purposes. The overall prevalence of E. coli from rectal samples 19.27%. In dogs, a variety of antimicrobials are used to treat common bacterial infections, including respiratory infection, urinary tract infections, wound infections, ear infections, gastroenteritis, and pyoderma. Resistance to these antimicrobials squeezes the treatment options of the companion animals. The E. coli isolates from dogs were found to be highly resistant to ceftriaxone (83.8 %). As ceftriaxone is also a widely used antibiotic in humans, it might be risky for humans in the context of AMR if these E. coli can somehow pass into humans by direct contact or from the environment [20]. Higher levels of resistance toward sulfamethoxazole/trimethoprim (62.1 %) ox tetracycline (54%) and Ampicillin (54%) were also observed in this study, which is higher than that reported by [21].

The E. coli isolates were highly susceptible to gentamicin 59(79.8%), erythromycin 48.6%, cefoxitin 36.5% ciprofloxacin 32.4%. This might be due to the less availability and utilization of these drugs in the country and study area. Susceptibility of E. coli isolates to erythromycin ciprofloxacin, cefoxitin and gentamicin could also be attributed to their inadequate utilization in canine clinical practice in Ethiopia. The lower level of antimicrobial resistance observed against erythromycin (33.8%), and gentamicin (16.2%) in the present study is in line with the 79% antimicrobial susceptible E. coli isolates reported previously[20]. Unlike the present study, increased detection of pathogenic and non-pathogenic E. coli that are resistant to antimicrobial drugs have been previously reported [22]. Dogs are generally the close companions of their human caretakers thereby providing opportunities for the exchange of antimicrobial-resistant bacteria.

Conclusion and Recommendation:

The present study showed that *E. coli* is common among apparently healthy and clinically ill dogs in Harar, a town in eastern Ethiopia. Moreover, the bacteria are isolated more frequently in clinically ill dogs than in apparently healthy dogs, indicating the possible role of *E. coli* in the GIT disease symptoms, and dogs might play a role in the spreading of the disease to humans as well as other animals. Based on the risk factor assessment, breed and age of dogs showed an association with *E. coli* presence, particularly in younger (< 6 months) and local breed dogs. Hence, it is important to consider the age of the dog; particular consideration must be given to the age of pet dogs in contact with vulnerable populations and in management practices. The other notable finding was the higher prevalence of *E. coli* in those households as well as dogs with offal as their main feed, which raises an alarm on the possible role of offal as a vehicle for *E. coli* transmission.

More importantly, because of the greatly increased risk of shedding pathogens like E. coli, raw meat and other raw animal products should not be fed to dogs within households of or in contact with vulnerable people. As well, these studies provide baseline data for comparison in future local, national, and international studies on the prevalence of these important zoonotic pathogens in pet dogs. Most of the E. coli isolates from dogs are susceptible to many of the drugs used in both human and veterinary medicine. However, some of the isolates have developed multidrug resistance and might be a potential source of the spread of antimicrobial-resistant E. coli from dogs to humans and other animals. Further, large-scale epidemiological studies including the contribution of dogs in the transmission to humans, serotyping and virulence gene detection of E. coli, frequent monitoring of antimicrobial drug resistance and responsible dog ownership, and improved hygienic management of dogs , anyone handling a pet or feces from a pet should wash their hands immediately afterwards with soap and running water or use an alcohol-based hand sanitizer, awareness campaign should be launched and provided on zoonotic canine diseases prevention measures and good hygienic practices, dogs should be strongly discouraged from eating their own feces or those of other animals, feeding a commercially prepared, heat-processed diet helps to reduce the risk of *E.coli* contamination in the food, but even these products can occasionally contain pathogen recommended

Availability of data and materials:

All necessary data supporting our findings can be found in this manuscript.

Conflicts of Interest:

The authors declare that they have no conflicts of interest in publishing this manuscript.

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