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Cross-Sectional Study

Assessment of Hygienic Practice, Isolation and Antimicrobial Susceptibility Test of *E. coli* from Honey Bees Farms in and Around Haramaya University and Haramaya Woreda, Ethiopia

Ahmedyasin M. Aliyi, MSc[1*]; Adem Hiko, DVM, MSc, PhD[2]; Abdallahi Abdureman, VLT, MSc[2]; Mohammedkemal M. Ame, MSc[3]

[1]*Department of Veterinary Public Health, College of Veterinary Medicine, Haramaya University, P. O. Box 138, Dire Dawa, Ethiopia*
[2]*Haramaya University, College of Veterinary Medicine, Ethiopia, P. O. Box 138, Dire Dawa, Ethiopia*
[3]*Department of Veterinary Public Health, Furda Veterinary Clinic, Bedeno Woreda, Eastern Hararghe, Ethiopia*

Corresponding author
Ahmedyasin M. Aliyi, MSc
Department of Veterinary Public Health, College of Veterinary Medicine, Haramaya University, P. O. Box 138, Dire Dawa, Ethiopia; **Tel.** +251910788420;
E-mail: ahmedyasinmohammed2018@gmail.com

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ABSTRACT

Aim
Ethiopia, a leading honeybee producer, has been impacted by a variety of pathogens, including viruses, bacteria, and parasites, leading to colony collapse.

Methods
A cross-sectional study was conducted from July 2022 to March 2023 to assess hygienic practices, isolate *Escherichia coli* from honey bees using feed swabs and hive swabs, analyze the antimicrobial susceptibility of *E. coli* isolated from honey bees, assess the hygienic and management practices of honey bee farms, and identify the major risk factors for E. coli presence in honey bee farms within the study area.

Results
Out of 225 samples collected, 42 (18.7%) were found to be *E. coli*, with higher prevalence in Tuji Gabisa (24%) and Damato (21.4%) kebele. The study also found that the highest resistance was found towards Ampicillin (78.6%) and Kanamycin (40.5%), followed by Erythromycin (31%), Tetracycline (30%), and Gentamicin (26.2%). Of the 42 isolates, 92.85% single- to multiple-drug-resistant isolates.

Conclusion
Beekeepers in the study area provided supplementary feed for honey during scarcity, and the most common locally available feed types were sugar, tea, and bore holly. The study serves as a baseline data for future research, highlighting the importance of addressing *E. coli* resistance and promoting hygienic practices.

Keywords
Antimicrobials, *E. coli*; Hive; Honey bee; Prevalence.

INTRODUCTION

Background

Since cultivated plants used to manufacture honey have become more prevalent over the past ten years, honey bee colonies have been dying off at higher rates. If honey bee hive colonies are developed to forage on these resources, it will be vital.[1] In Ethiopia, it is anticipated that exports of honey and beeswax generate annual export revenues of 1.6 million USD on average.[2] Beekeeping contributes significantly to socioeconomic growth and naturally eliminates pollination and conservation.[3] Ethiopia has an abundant natural resource base for the production of honey, and beekeeping is a long-

standing household activity in practically all regions of the nation. Nonetheless, the sector's contribution to the country and beekeepers is not acceptable.[4]

A communal immune response known as hygienic behavior has been found to assist honey bees in resisting pests and diseases that affect their hives.[5] Resistance to diseases and pests that cause honey bee colonies to lose their workers can be gained through hygienic behavior.[6] The importance of natural materials as medications or drug leads for human health cannot be overstated.[7] The greatest effect on human health has come from their use as antibiotics to treat infectious diseases.[8]

Honey is one alternative medical therapy that has recently attracted a lot of attention. Apis mellifera and Meliponinae honeybee species create honey.[9] From a variety of sources, its antibacterial effectiveness varies significantly with processing and source, based on the naturally occurring vegetative blooms blooming in various seasons and locations.[10] Particularly commensal intestinal bacteria serve as gene repositories for antibiotic resistance.[11] Commensal *Escherichia coli* (*E. coli*), the most frequent bacterial pathogen causing foodborne illness, can pass on antibiotic resistance to other Enterobacteriaceae, including several Salmonella serovars, in the gastrointestinal environment, especially when antimicrobial exposure occurs.[12]

Statements of Problems

The aggressiveness, propensity for swarming, and absconding behaviors of bees, a lack of skilled labor and training facilities, low technology usage, high cost of improved beekeeping technologies, drought and deforestation of natural vegetation, inadequate postharvest management of beehive products and marketing restrictions, indiscriminate application of agrochemicals, and honeybee disease are the main obstacles in Ethiopia's beekeeping subsector.[13] Yet, not every region of the nation may be affected by all of these issues, nor may they all be equally urgent in every location.[14] Recognized and prioritized the production's biggest issues.

A major worldwide health concern, antimicrobial resistance (AMR) will have a significant impact on future treatment and prevention of infections in humans and honey bees, as well as on our ability to treat resistant illnesses in human and veterinary medicine.[15,16] On the other hand, erythromycin and ampicillin showed the highest levels of resistance, followed by tetracycline, co-trimoxazole, and amoxicillin-clavulanic acid.[17] Previous research in Iran found that the above antibiotics had the highest efficacy and the highest rates of resistance.[18] The goals of honey bee production, health management techniques, and limiting factors are poorly known at Haramaya University (HU). However, before settling on any planned application for honeybees, an examination of management techniques and the limitations of a lack of emphasis on government production is essential in order to make honeybee farm activities more efficient and sustainable.[19]

Moreover, these studies failed to provide detailed information on the risk factors for honey bee *E. coli*, and there is limited information on the antimicrobial susceptibility profiles of honey bee gut isolates. To the best of researchers' knowledge, there is no such study conducted in a study area as well as at the country level in general. Additionally, the data from this study were used as a preliminary study to provide baseline data for future research to explore pathogenic bacteria found in the gut, feed, and swab of honey bees. Therefore, the study was conducted with the objective of "*Assessment of Hygienic Practice, Isolation, and Antimicrobial Susceptibility Test of E. coli from Honey Bees Farms in Haramaya University and Haramaya Woreda. Eastern Hararghe, Ethiopia.*"

OBJECTIVES OF RESEARCH

General Objectives

• To quantify the level of hygiene practice, isolate E. coli, and test it for antibiotic susceptibility at honey bee farms in Haramaya University and Haramaya woreda Eastern Hararghe, Ethiopia.

Specific Objectives

• To isolate of E-coli from honey bee, feed swab and hive swab in the study area.
• To analyze the antimicrobial susceptibility of E. coli from honey bees in the research area's.
• To assess of hygienic and management practice of honey bee farms in the study area.
• To identify major risk factor of E.coli from honey bee farms in the study area.

MATERIALS AND METHODS

Study Area Description

The study was conducted at Haramaya University's and Woreda honeybee farms. Haramaya University and Woreda (Figure 1) are located in the eastern part of Hararghe Zone, Oromia regional state, Ethiopia; 509 km from Addis Ababa, the capital city of Ethiopia; 17 km from Harar; and 40 km from Dire Dawa. Haramaya University, Damota, and Tuji Gabisa are far apart from Haramaya town—5 km, 7 km and 8 km, respectively. It lies between 9026' N latitude and 4203' E longitude, 2000 m above sea level. The monthly average maximum and minimum temperatures of the area are 10 °C and 18 °C, respectively, and the area receives an annual rainfall of 800 mm.[20]

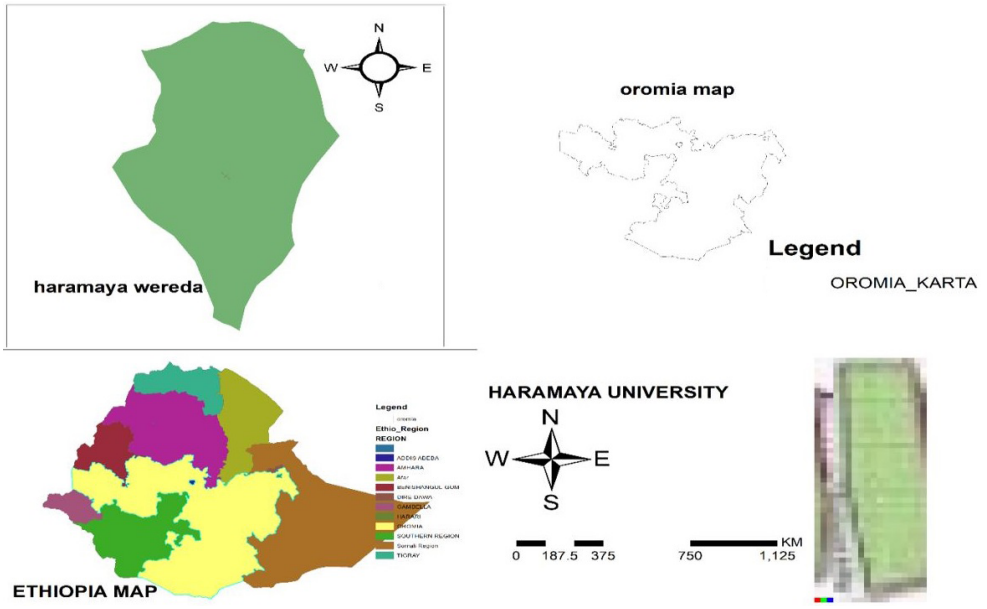
Study Population

The honey bee's farm was kept in both traditional and modern hives at Haramaya University and Woreda.

Study Design and Sampling Techniques

A cross-sectional study was conducted from July 2022 to March 2023 to assess hygienic practices, isolate, identify, and conduct anti-

Figure 1. *Map of the Study Area*



Source: from GIS

microbial susceptibility tests of *E. coli* from the gut of honey bees, feed, and hive swabs in the study area from those managed under traditional and modern beekeeping apiculture. Purposive sampling was used to collect the sample from the apiculture digestive gut of honey bees, feed, and hive swabs.

Sample Size Determination

A total of 225 samples (75 from digestive gut samples of honey bees, 75 feed swabs, and 75 hive swabs) were collected from the selected sites (75 from, Haramaya University apiculture, 75 from Damota, and 75 from Tuji Gabisa) proportionally.

Questionnaire Survey

A total of 40 respondents were interviewed from selected farms for the study to assess the hygienic and management practices of honey bee farms in the study area. The questionnaire, which took around 20 minutes to complete, was written in English, and interviews were carried out with the assistance of an employee at the Center for Livestock Farm.

Sample Collection Techniques

Three samples were collected from each hive included in the study. Accordingly, a total of 225 samples were collected from honeybees, feed, and hive swabs in the study area. Healthy adult worker honey bees were collected from each location in the early morning. Samples were collected from honey bees' swabs fed using sterile scissors and protective clothing and then labeled by type of hive from respective sites. Honey bee specimens were kept in plastic containers or tubes with perforated lids and small sugar-laden cake pieces. The collected feed and hive swabs were also collected aseptically in a sterilized test tube that contained peptone water. Subsequently, the samples were conveyed to the Microbiology Laboratory at the College of Veterinary Medicine, Haramaya University, in an ice box. Should immediate inoculation of the samples onto media prove inconvenient, they were stored at +4 °C until they could be processed for isolation.

Isolation and Identification of *E. coli*

Isolation and identification were performed according to the guidelines. The media used for isolation were MacConkey (MC) agar and eosin methylene blue (EMB) agar. The suspected colony was cultured on sorbitol MacConkey (SMC) agar (Oxiod). The media were prepared according to the manufacturer's instructions.

Isolation

A loop full of the enriched sample (a sample in buffered peptone) was spread on MC agar and incubated at 37 °C for 24 hours. Five representative typical colonies (pink colonies) from MC agar were then transferred to EMB agar and incubated at 37 °C for 24 hours. The organisms showing the characteristic colony morphology of *E. coli* were repeatedly subcultured on EMB agar until a pure culture with homogenous colonies was obtained. Then, isolated colonies showing a metallic sheen on EMB agar were transferred to nutrient agar for further biochemical characterization and morphological examination. The suspected colony from EMB to nutrient agar was cultured on SMC agar for pathogenic identification of *E. coli.*

Identification

Honey bee farms of suspected *E. coli* were subjected to Gram's staining to observe cellular morphology and Gram's reaction.

Furthermore, suspected isolates were tested for catalase reaction, oxidation-fermentation (OF), oxidase, indole production, methyl red (MR), vogues–proskaure (VP) reaction, citrate utilization, and motility. Generally, *E. coli* was considered gram-negative, motile, catalase-positive, fermentative, oxidase-negative, indole-positive, MR-positive, VP-negative, and citrate-negative.

Antimicrobial Susceptibility Test on *E. coli*

Overall, 42 bacteria isolates are resistant to the antimicrobial tests available for ampicillin, tetracycline, gentamicin, erythromycin, kanamycin, and vancomycin. Resistance to *E. coli*, which is associated with this disease, was found in all isolates tested.

Data Management and Analysis

Data from the questionnaire responses is entered into Microsoft Excel 2013 spreadsheets following the interviews and exported after the laboratory work is finished. Statistical analyses are conducted in statistics and data (STATA) software version 16. Descriptive statistics are computed to define farm characteristics and to determine knowledge, attitudes, and hygiene practices among respondents regarding the use of antimicrobials. Univariable logistic regression and Chi-square (χ^2) tests were used to examine possible associations between farm sizes, hygiene practices, feeding systems, and management factors, such as routines related to antimicrobial use.

RESULTS

Occurrence of *E. coli*

An overall prevalence of 18.7% *E. coli* was observed in 225 samples collected from honey bees, feed, and hive swabs in the study area (Table 1). Except for the sample source, which shows a significant difference in prevalence at χ^2=9.133 with a *p*-value of 0.010, other variables show non-significance. On the other hand, it was relatively high at Tuji Gabisa (24%) by Keble and in Traditional (20.33%) with no significant difference.

The prevalence was higher in Tuji Gabisa (24%), as compared with the other Kebele. The prevalence was higher in Tuji Gabisa (29.6%) and Damato (21.4%) kebles in modern and traditional hive types, respectively, than in other kebele. The chi-square analysis indicated that the differences observed were statistically significant (p<0.05) in the prevalence of *E. coli* among the studied Kebele (Table 2).

Table 1. *An Overall Prevalence of E. coli in the Studied Area*

Study Variables		Tested sample N. (%)	Number of Positive	% Positive
Location	Haramaya University (HU)	75	10	13.3
	Tuji Gabisa kebele	75	1 8	24
	Damota Kebele	75	14	18.7
Hygiene level	Modern	102	17	16.67
	Traditional	123	25	20.33
Sample source*	Honey bee	75	12	16.0
	Feed	75	22	29.3
	Hive swab	75	8	10.7
Total		225	42	18.7

Note: Significance was observed only for sample sourceat χ2 =9.133; p-value 0.010

Table 2. *Prevalence of E. coli by Hive Type among Study Areas with Related Hive Type*

Keble	Hive Type	Tested Sample N (%)	Number of Positive	% Positive	χ2	*p* value
HU	Traditional	11(33)	6	18.2	1.19	0.274
	Modern	14(42)	4	9.5		
	Total	25(75)	10	13.3		
Tuji Gabisa Kebele	Traditional	16(48)	10	20.8	0.733	0.392
	Modern	9(27)	8	29.6		
	Total	25(75)	1 8	24		
Damota Kebele	Traditional	14(42)	9	21.4	0.480	0.489
	Modern	11(33)	5	15.2		
	Total	25 (75)	14	18.7		
Total		225	42	18.7		

Key: *χ2 value is a comparison among the study sites, N=number of sampled hives, n=number of Sampled, %=percentage, and p-value*

Table 3. *E. coli Isolated from the Gut of Honeybee, Feed, and Hive Swab in the Study Area*

Keble	Sample Point	No. of Sampled	No. of Positive (%)	$\chi 2$	*p* value
HU	Honey bee	25	3(12)	1.615	0.446
	Feed	25	5(20)		
	Hive swab	25	2(8)		
Tuji Gabisa Kebele	Honey bee	25	4(16)	8.33	0.016
	Feed	25	11(44)		
	Hive swab	25	3(12)		
Damota Kebele	Honey bee	25	5(20)	1.230	0.541
	Feed	25	6(24)		
	Hive swab	25	3(12)		
Total		225	42(18.7)		

Key: *$\chi 2$=value is a comparison among the study sites, and p-value*

The study revealed that the prevalence of *E. coli* was higher in the feed sample (20%) as compared with other samples examined from HU. Moreover, the prevalence of feed samples was higher in Tuji Gabisa Kebele (44%), compared with other Kebele (Table 3).

Antimicrobial Susceptibility Profiles of Isolates

The AMR profile is outlined in Table 4. In the cases of *E. coli*, the highest resistance was found toward ampicillin (78.5%) and kanamycin (40.5%), followed by erythromycin (31%), tetracycline

Table 4. *Antimicrobial Susceptibility Profile of E. coli Isolates*

Antimicrobial Drug (agent)	Disc Content (µg)	Susceptible No. (%)	Intermediate No. (%)	Resistant No. (%)
Tetracycline	30	21(50)	8(19)	13(30)
Gentamicin	10	25(59.5)	6(14.3)	11(26.2)
Erythromycin	15	7(16.7)	22(52.4	13(31.0)
Vancomycin	30	27(64.3)	8(19.0)	7(16.7)
Kanamycin	30	11(26.2)	14(33.3)	17(40.5)
Ampicillin	10	4(9.5)	5(11.9)	33(78.6)

(30%), and gentamicin (26.2%). Only 16.7% of the isolates showed resistance to vancomycin.

Below, Table 5 shows the distribution and patterns of single- to multiple-drug resistance isolates. Of the total 42 isolated, 39 (92.85%). One drug, two drugs, three drugs, four drugs, and five drugs resistant were frequent for AMP (11.9%), AMP-KAN (9.52%), AMP-GEN-ERY (4.76%), TT-ERY-KAN-AMP (2.38%), and TT-GEN-KAN-AMP-ERY (2.38%), respectively.

Assessment of Hygienic of Bee Farm in the Study Area

Of the beekeepers in the study area of the participants, 85% said that they provided supplementary feed for honey during the scarcity of feed. The most common locally available feed types used for colony supplements identified were sugar, tea, and no, as explained in Table 6. Additionally, honeybees collect water from 35% lakes, 35% tap water, and 27.5% bore holly, as indicated in Table 3. Sample respondents were asked whether they received training concerning the techniques of beekeeping and its management. The respondent, management, and variables were all statistically significant risk factors.

DISCUSSION

The health of honeybees has been one of the most important topics in apiculture research in recent years. Honeybee diseases are considered major afflicting factors for honeybee health, and huge colony losses are linked to these diseases. This is mainly associated with the recent emergence of high honeybee colony losses in many parts of the world. Consequently, disease control is a very challenging task for protecting honeybee populations. At present, honeybee disease control mainly depends on antibiotics such as tetracycline. However, using antibiotics in apiculture is legally banned in many countries of the European Union because of the risks they present for both human and honeybee health. The use of antibiotics may also lead to the emergence of resistant bacterial strains.

The overall occurrences of *E. coli* in honey bee gut, feed, and hive samples from study areas were 18.7%. The present study

Table 5. *Distribution and Patterns of Single to Multiple Drug Resistance E. coli Isolates*

No. of Drugs Resistant	Resistant Drug Patter	No. of Isolated Resistant	Percentage (%)
One drug	AMP	5	11.9
	KAN	2	4.76
	ERY	2	4.76
Two drugs	TT, AMP	3	7.14
	AMP, KAN	4	9.52
	VAN, AMP	2	4.76
	GEN, AMP	1	2.38
	AMP, ERY	3	7.14
Three drugs	AMP, VAN, KAN	1	2.38
	AMP, GEN, ERY	2	4.76
	AMP, TT, KAN	2	4.76
	TT, VAN, KAN	1	2.38
	KAN, AMP, ERY	2	4.76
	GEN, KAN, AMP	2	4.76
	GEN, TT, KAN	1	2.38
	TT, VAN, AMP	1	2.38
	TT, GEN, AMP	1	2.38
Four drugs	TT, ERY, KAN, AMP	1	2.38
	GEN, VAN, AMP, ERY	1	2.38
Five drugs	TT, GEN, KAN, AMP, ERY	1	2.38
	TT, VAN, GEN, ERY, AMP	1	2.38
None resistant		3	7.14
Total		**42**	**100**

Keys: AMP=Ampicillin; ERY=Erythromycin; GEN=Gentamicin; KAN=Kanamycin; TT= Tetracycline; VAN= Vancomycin

Table 6. *Cleaning, Supplement Feed, training, status of Colony Collapse, Frequency of Cleaning Equipment of Honeybee E. coli Positive Risk Factors*

Management	Category	N	%	χ^2	*p* value
Do you Provide supplement feed?	Yes	34	85.0	681.9	0.000
	No	6	15.0		
Type supplement	Sugar	19	47.5	681.9	0.000
	Tea	15	37.5		
	No	6	15.0		
Type of water	Bore holly	12	30	681.9	0.000
	lake	14	35		
	Tap	14	35		
Frequency of cleaning equipment	Every week	9	22.5	681.9	0.000
	Every monthly	12	30		
	No	19	47.5		
Do you get beekeeping training?	Yes	33	82.5	681.9	0.000
	No	7	17.5		
Have ever encountered colony collapse?	Yes	30	75	681.9	0.000
	No	10	25		
	Total	**40**	**100**		

Key: N=number of respondents, n=number of positive isolates, %=percentage-value, $\chi 2$ =value is comparison among the type risk factors

disagrees with a study conducted on the prevalence of Klebsiella in the guts of bees in and around Haramaya University Bee Farm, East Hararghe, and Oromia Regional State, Ethiopia, that reported 50% of Klebsiella. This is inconsistent with the study conducted by Raymann et al[21] and Khan et al[22] that observed the occurrence of multiple Enterobacteriaceae in the gut of bees, including *E. coli*,

Klebsiella, and other bacterial species. Additionally, this study was similar to that of other researchers Bog et al[23] who identified *E. coli* in honey bee guts.

The frequencies of bacterial species isolated according to Keble were 10 (13.3%), 18 (24%), and 14 (18.7%) for HU, Tuji Gabisa, and Damota, respectively. The study conducted in Riyadh and AI Baha that reported about 36.4 and 45.5% of the gut microbiomes in bees (24) was higher than the current study. The prevalence of *E. coli* was higher in Tuji Gabisa (24%), as compared with the other Kebles. The prevalence was higher in Tuji Gabisa (29.6%) and Damato (21.4%) Kebele in modern and traditional hive types than other Kebles, respectively. The current study was contradicted by a study carried out around HU[20] that reported 50% of Klebsiella in both hive types (modern and traditional). Also, the current finding was lower than that reported in Mexico, which reported about 38%. The *E. coli* isolate was higher in the modern hive type (75% of the total) than in the traditional type. Chi-square analysis revealed that there were statistically significant differences ($p<0.05$) in the prevalence of *E. coli* among the studied Kebles (Table 1). This difference might be due to poor management practices as compared with other sites that depend on observation and interview data. However, to compare with other studies, to my knowledge, there is no report on the isolation of *E. coli* from honeybees in Ethiopia or Africa yet.

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, the use of antimicrobials as growth promoters, as well as the use of antimicrobials commonly used in human practice.[24] Antimicrobial resistance has been suggested as an important therapeutic problem in veterinary and human medicine.[25]

In this study, the antimicrobial resistance of *E. coli* isolated from honeybees was investigated against six antimicrobial drugs using the disk diffusion method. Overall, moderately low antimicrobial resistance was found (16.7%). Antibiotic sensitivity patterns might explore the possible multidrug resistant (MDR) bacteria in honeybees, and it may cause problems in humans if they are infected with MDR bacteria from honeybees. The findings of the study revealed the common bacterial pathogens circulating in honeybees and also showed their extended spectrum of resistance to several antibiotics that are commonly used for therapeutic purposes.

In the present study, *E. coli* isolates were resistant to tested antibiotics. The highest resistance was found toward ampicillin (78.6%) and kanamycin (40.5%), followed by erythromycin (31%), tetracycline (30%), and gentamicin (26.2%). Only 16.7% of the isolates showed resistance to vancomycin. The high resistance of these drugs in gram-negative bacteria might be due to the transfer of resistance genes from gram-positive bacteria, including β-lactamase genes.[26] The present study was opposite the work of the previous researchers,[27] who tested 42 isolates of *E. coli* isolated from the digestive tracts of honey bees for several antibiotics and determined low-level resistance to gentamicin and chloramphenicol. Meanwhile, they detected the highest level of antibiotic resistance for kanamycin. The distribution and patterns of single- to multiple-drug resistance isolates. Of the total 42 isolates, 39 (92.85%) The present finding is similar to the 39 (92.85%) recorded in the previous study.[28]

The development of antimicrobial resistance by the bacteria to these drugs poses a major challenge in both human and animal medicine because these drugs are commonly used in the treatment of human patients and veterinary practice. Antimicrobial resistance of *E. coli* isolates from animal and human sources has been reported in Ethiopia.[29] So, disease control is a very challenging task for protecting honeybee populations. To reduce the risk of disease management practices like honeybee equipment washing as well as improving the water supply, sanitary conditions in apiculture are very important, and you should also replace traditional hives with modern ones. Because modern hives make it simple to inspect conies, control disease, feed, and maintain apiculture sanitation.

Of the beekeepers in the study area of the participants, 85% said that they provided supplementary feed for honey during the scarcity of feed. The most common locally available feed types used for colony supplements identified were sugar, tea, and hay, as explained.[30] Additionally, honeybees collect water from 35% of lakes, 35% tap water, and 27.5% bore holly, as indicated in different sources from me, and 60% provide supplementary carbohydrates and proteins in the western Amhara region.[31] Sample respondents were asked whether they received training concerning the techniques of beekeeping and its management. Accordingly, the majority of workers, or 82.5% of the interviewed beekeepers, had gotten beekeeping training from Haramaya University, as explained in Table 4.

Beekeepers in the study area provided supplementary carbohydrate feed for honeybees during feed scarcity, which was higher than 3.1% in the Haramaya district.[30] Like other insects, honeybees have 33 symbiotic and pathogenic interactions with microbes in their digestive tracts,[32] which are assumed to be influenced by the environment where they find food. To overcome the problem, supplementary feeding is required for the honeybees. The majority of the honey bee attendants (85%) have attended beekeeping training from Haramaya University and livestock experts on production management, which is higher than the provisional reported by Yeserah[33] from Haramaya Woredas and Haramaya University due to the ease with which they got training on beekeeping from researchers and livestock experts in Haramaya town. Moreover, beekeeping training develops the beekeepers, increasing sanitation and disease control management.

CONCLUSION AND RECOMMENDATION

In this investigation, the occurrence of *E. coli* was evaluated in honey bees, feed, and hive swabs from the study area. *E. coli* was found in 42 samples (18.7%) of the total. In other words, the highest isolates were for *E. coli* from Tuji Gabisa (24%), as compared with the other Kebele. The occurrences of *E. coli* isolates (20-44%) among

honeybee feed in the study by Keble indicated a high distribution of *E. coli* present in the honeybee feed in the hives. Moreover, this shows that honeybee feed, gut, and hive swabs are alternate habitats for human pathogenic bacteria. The types of hives in the study were Tuji Gabisa (29.5%) and Damato (21.4%). Keble in modern and traditional hive types, respectively, and management are identified as risk factors for the presence of *E. coli* in honeybees. However, a statistically significant difference was observed between these sites of study, and all assessment risk factors ($p<0.05$) for isolates that were fed supplements, type of water used, and colony collapse were significantly associated ($p<0.05$). It was evidence of a low-level of public awareness about bacterial honeybee disease and its associated risk factors in the study area. Additionally, the present finding revealed that the isolated organisms were tested for antimicrobial susceptibility patterns for ampicillin, kanamycin, erythromycin, tetracycline, gentamicin, and vancomycin. The results of this test indicated that all isolated bacterial species were fully resistant to ampicillin, kanamycin, erythromycin, tetracycline, and vancomycin. It is concluded that *E. coli* can contaminate the honeybee feed, where it might colonize the honeybee gut, with the risk of human infection. In line with the findings, the following recommendations were forwarded:

- The use of a modern hive instead of a traditional hive is advisable due to the ease of management practices like inspection, feeding, sanitation, and disease control.
- Great emphasis should be given to honeybee health training and extension programs for the community.
- Focusing on the practical aspects of general beekeeping, and more specifically on honeybee
- The management of hygienic conditions in apiculture is important.

DECLARATIONS

Ethics approval and consent to participate even though the study subjects (honey bees farm) were not exposed to damages due to the nature of the sample (swab sample), the best practice guidelines for veterinary care were applied during sampling. Because our study didn't include animal experimentation, our study was not subjected to ethical review by the "*Animal Research Ethical Review Committee of Haramaya University, College of Veterinary Medicine.*"

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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