Occurrence and antimicrobial susceptibility of *Salmonella* in feces and milk samples of lactating dairy cows in Addis Ababa, Ethiopia

Habtamu Mekonnen[1], Haile Alemayehu[2], Musse Girma[2], and Tadesse Eguale[2]*

[1] *Addis Ababa Urban Agriculture Development Commission, Addis Ababa, Ethiopia, email: meha451@gmail.com*

[2] *Aklilu Lemma Institute of Pathobiology, Addis Ababa University, P.O.Box 1176, Addis Ababa, Ethiopia. Email: haile.alemayehu@aau.edu.et*

[2] *Aklilu Lemma Institute of Pathobiology, Addis Ababa University, P.O.Box 1176, Addis Ababa, Ethiopia. Email: mussegirma@yahoo.com*

[3] *Aklilu Lemma Institute of Pathobiology, Addis Ababa University, P.O.Box 1176, Addis Ababa, Ethiopia. Email: tadesse.eguale@aau.edu.et*

***** *corresponding author*

Abstract

Salmonella is one of the major causes of foodborne diseases that remained important public health concerns worldwide. Antimicrobial resistance in *Salmonella* is also a global concern. Establishing the status of *Salmonella* in dairy farms and antimicrobial susceptibility of circulating isolates particularly where animals and humans live in close proximity is vital to devise appropriate intervention. A cross-sectional study was conducted from December 2019 to May 2020 to determine the prevalence of *Salmonella* and antimicrobial susceptibility of isolates among lactating dairy cows in Addis Ababa. A total of 151 fecal and 151 milk samples were collected from lactating dairy cows and cultured for *Salmonella*. *Salmonella* isolation and identification was conducted using standard microbiological techniques and further confirmation was carried out using *Salmonella* genus-specific PCR. An antimicrobial susceptibility test was performed using the Kirby-Bauer disk diffusion technique. *Salmonella* was isolated from 4 fecal samples (4/151) (2.7%) whereas none of the 151 milk samples were positive for *Salmonella*. One isolate was multidrug-resistant (MDR) to seven antimicrobials namely: ampicillin, amoxicillin, ceftriaxone, cephalothin, tetracycline, and sufamethoxazole+ trimethoprim and two isolates were resistant to either tetracycline or sulfisoxazole. All *Salmonella* isolates were susceptible to ciprofloxacin, amikacin, chloramphenicol, nalidixic acid, and gentamicin. In conclusion, a low prevalence of *Salmonella* among lactating dairy cattle was recorded in this study and it was not detected in milk

samples. However, the observed resistance to commonly used antimicrobials particularly third-generation cephalosporin, ceftriaxone in one of the isolates pose a public health concern. Thus, appropriate measures should be instituted to protect the public and animals from infection with multidrug-resistant strains of *Salmonella*.

Keywords: Addis Ababa; dairy cows; antimicrobial resistance; prevalence; *Salmonella*.

Introduction

A large segment of the rural and urban population of Ethiopia is dependent on livestock for food and generation of income and animals and humans live in close proximity most of the time. Thus, many zoonotic bacterial pathogens can reach humans through the consumption of contaminated foods of animal origin and close contact with animals infected by these pathogens (Bekele and Ashenafi, 2010).

Foodborne diseases originating from food animals remain major public health problems across the globe where healthy food animals serve as reservoirs of pathogens spreading to humans (Eng *et al*., 2015). An estimated 600 million cases, 420,000 deaths, and 33 million years of healthy lives are lost due to the consumption of unsafe food globally each year (WHO, 2015). The common foodborne pathogens are zoonotic and transmitted through the consumption of contaminated foods of animal origin or direct or indirect contact with the animals (Rahman *et al*., 2020).

Salmonella is one of the major foodborne pathogens that cause diseases ranging from mild gastroenteritis to severe complications including invasive bloodstream infections. Human infection with *Salmonella* occurs mainly when a person consumes food of animal origins such as meat, poultry, dairy products, and other products contaminated with the feces of animals containing *Salmonella* (Bintsis, 2017). Cattle have been identified as a major reservoir of *Salmonella* and shed the bacteria in feces leading to contamination of milk and other dairy products providing an easy route of human infection with *Salmonella* (Eguale *et al*., 2016; Holschbach and Peek, 2018). Both symptomatic and asymptomatic dairy cattle are potential sources for the contamination of the farm environment and farm products by *Salmonella* species (Cummings *et al*., 2010).

Consumption of unpasteurized milk and undercooked meat contaminated with fecal material are the main transmission routes for *Salmonella* in humans (Bintsis, 2017). Antimicrobial resistance in *Salmonella* has been considered the major public health problem globally. Infection with antimicrobial-resistant *Salmonella* is reported to be associated with treatment failure, increased risk of bloodstream infection, increased chance of hospitalization, and prolonged hospital stay (Krueger *et al*., 2014; Varma *et al.*, 2005).

Increasing antimicrobial-resistant *Salmonella* are being reported in different parts of the world due to the use of antimicrobial agents in food animals at subtherapeutic level or prophylactic doses which may promote on-farm selection of antimicrobial-resistant strains and markedly increase the human health risks associated usually with the food supply (Cheng *et al*., 2019; Manyi-Loh *et al*., 2018).

In Ethiopia, various studies showed the occurrence of *Salmonella* in dairy cattle and their environment (Addis *et al*., 2011; Eguale *et al*., 2016; Keba *et al*., 2020). Although the contribution of different food animals as a source of *Salmonella* infection to humans is not clearly understood, a study showed a clear indication that related genotypes of *Salmonella* are circulating among humans and animals particularly, multidrug-resistant *Salmonella* Kentucky in dairy cattle and diarrheic patients in Addis Ababa (Eguale *et al*., 2018). Due to the increasing demand for milk and other dairy products for the rising human population of Addis Ababa, small-scale dairy production is rising in Addis Ababa and its suburbs (Eguale *et al*., 2016; Minten *et al*., 2020). The majority of these dairy farms are located near human residential areas increases the chance of spreading *Salmonella* and other pathogens from animals to humans. In addition, as the consumption of raw milk and its derivatives is common in Ethiopia (Keba *et al*., 2020) there is a high chance of infection with *Salmonella* and other foodborne pathogens.

As there are few studies conducted in Ethiopia on the prevalence of *Salmonella*, limited information is available on the level of its occurrence in lactating dairy cows and milk from cows and antimicrobial susceptibility of isolates in Addis Ababa. Hence, establishing the current status on the prevalence and antimicrobial susceptibility of *Salmonella* in the feces of lactating cows and corresponding milk samples is critical for the control of *Salmonella* isolates originating from dairy farms. Therefore, the purpose of this study was to estimate

the prevalence of *Salmonella* species in feces and milk samples of lactating dairy cattle from selected dairy farms in Addis Ababa and also to determine the antimicrobial susceptibility of *Salmonella* isolates obtained from collected samples.

Materials and methods

Study area, design, and animals

This study was conducted in Addis Ababa, the capital city of Ethiopia, from December 2019 to May 2020. Addis Ababa is located at 9°148 N38°44 24E coordinate and an average elevation of 2355m above sea level. The average annual temperature and rainfall are 21°C and 1800 mm, respectively. Addis Ababa, with a 527 square kilometer area, is composed of ten sub-cities namely Addis Ketema, Arada, Gulele, Kirkos and Lideta, Akaki Kality, Bole, Kolfe Keranio, Nifas Silk Lafto, and Yeka when this study was conducted. The population of Addis Ababa is estimated at 4.7million (COI, 2020). The dairy farms in urban and peri-urban Addis Ababa are the source of milk for the community in Addis Ababa supplying almost one-third of all liquid milk consumed in the city (Minten *et al*., 2020).

A cross-sectional study was conducted to determine the prevalence and antimicrobial susceptibility of *Salmonella* among lactating dairy cows in Addis Ababa. The study was conducted on selected dairy cows from small, medium, and large-scale dairy farms. Farms were categorized into small (those containing 5–20 animals in a herd), medium (21–50 animals in a herd), and large (more than 50 animals). Clinically healthy lactating cows were involved in this study.

Sample size determination

The larger prevalence report of previous studies on *Salmonella*from lactating dairy cattle in Ethiopia 10.76% (Addis *et al*., 2011) was used to calculate sample size using the formula given by Thrusfield (2007). Accordingly, the minimum sample size calculated was 138, and13 samples were added to improve precision. Therefore, a total of 151 lactating dairy cows were involved from which corresponding151 fecal and 151 milk samples were collected from a total of 47 dairy farms of different herd sizes.

Sampling method

Among the ten sub-cities of Addis Ababa, Arada, Akaki Kality, Kirkos, Nifas Silk, and Yeka sub-cities were randomly selected. Then based on the list of the name of the farm owners in the sub-cities obtained from the Addis Ababa Farmers and Urban Agriculture Commission, a systematic sampling technique was used to determine the farms involved in the study. The majority of the farms were small scale having herd sizes of not more than six cows. All lactating cows from each farm were sampled except for the medium and large-scale dairy farms from which the maximum of 10% of lactating cows was sampled.

The study inclusion criteria for the farms was based on a farm with at least 5 Holstein-Friesian-zebu cross breed cattle and 1 lactating cow during the study time, willingness to participate in the study, ready to give the required samples and information through the questionnaire. Cows that received or receiving antimicrobials during the last 10 days were excluded.

Sample and data collection

A total volume of 40ml of milk samples were collected in sterile 50 ml containers (Falcon tubes) directly from the teats and 10 gm of fecal samples were collected directly from the rectum using disposable gloves into clean zippered plastic bags and packed to avoid any possibility of leakage or cross-contamination. Collection of milk was done by farm attendants by first cleaning his/her hands and the udders and teats with clean water. This was done following their routine milking schedule of each farm where milk samples were collected in the middle of milking of each cow. A minimum of one cow and a maximum of 5 cows were sampled per farm depending on farm size and availability of lactating cows during the study period. Samples were coded properly and transported to the Microbiology Laboratory of Aklilu Lemma Institute of Pathobiology, in an icebox within 3-4 hrs of collection and processed directly for isolation of *Salmonella*. Information on possible risk factors and socio-demographic data of each selected farm was also gathered using a structured questionnaire. The questionnaire includes sex, age, educational status of the farm owners and attendants, frequency of barn cleaning, age of cows, parity, types of bedding, milking process, and antimicrobial use. Collection of data was performed at the time of sample collection from each farm.

Isolation of *Salmonella*

Salmonella isolation was conducted at the Microbiology Laboratory of Aklilu Lemma Institute of Pathobiology, Addis Ababa University using techniques recommended by International Organizations for Standardization (ISO-6579, 2000) and WHO Global Foodborne Infections Network laboratory Protocol (WHO, 2010). Ten g of fecal sample and 10ml of milk was pre-enriched in 90 ml of buffered peptone water (BPW) (Oxoid CM509, Basingstoke, England), and incubated for 24 hrs at 37°C. A portion (1 ml) of the pre-enriched culture suspension was transferred to 10ml of selenite cysteine (SC) (Himedia M025, India) broth and another 0.1 ml portion was transferred to 10 ml of Rappaport and Vassiliadis (RV) (Merk, Germany) enrichment broth and incubated at 37°C and 42°C for 24 hrs, respectively. Samples were then inoculated onto Xylose Lysine Deoxycholate (XLD) (Oxoid CM0469, Basingstoke, England) agar plate from both selective enrichment broth media and incubated at 37°C for 24 hrs and the incubation was prolonged to 48 hrs when there were not clear distinct colonies during the 24 hrs of incubation. Presumed *Salmonella* colonies having a slightly transparent zone of reddish color and a black center (WHO, 2010) were sub-cultured on nutrient agar (OxoidCM0003, Basingstoke, England) and further assessed using biochemical tests: triple sugar iron agar (TSI) (Oxoid CM0277, Basingstoke, England), urea (Oxoid CM53, Basingstoke, England), lysine iron agar (LIA) (Oxoid CM381, Basingstoke, England), and Simmons citrate agar (Becton Dickinson, USA) as described previously (Eguale *et al*., 2016). The isolated colonies with typical *Salmonella* biochemical properties were then further confirmed by genus-specific polymerase chain reaction (PCR) as described previously using primer sets (Cohen *et al*., 1993). Two to three pure colonies grown overnight on XLD agar were suspended in ultra-pure RNA's free water and boiled for 5 minutes at 95°C in thermocycler which was used as a DNA template for PCR assay. A reference strain of *S.* Typhimurium (ATCC 14028) was used as a positive control during biochemical analysis and PCR assay.

Antimicrobial susceptibility

The antimicrobial susceptibility profiles of isolates were determined by the Kirby-Bauer disc diffusion method on Muller-Hinton agar (Oxoid CM0337 Basingstoke, England) according to Clinical and Laboratory Standard Institutes Guidelines (CLSI, 2018). Pure colonies grown overnight on nutrient agar were inoculated to a tube containing 5 ml of normal saline solution until it achieved 0.5 McFarland turbidity standards. When the turbidity of bacteria

was higher than that of 0.5McFarland standard, it was diluted using sterile saline solution. The sterility of the Muller Hinton agar plate was checked by incubating overnight at 37º C before using it for an antimicrobial susceptibility test. A sterile cotton swab was dipped into the suspension and the bacteria were swabbed uniformly over the surface of the Muller Hinton agar plate after removing excess fluid on the cotton swab by pressing on the whole of the test tube. Antimicrobial discs with known concentrations were placed and held at room temperature for 15 minutes to allow drying. The list of antimicrobial discs used in this study, their strengths, and clinical cut-off points are shown in Table 1. All antimicrobial discs used in this study were from Sensi-Discs, Becton, Dickinson, and Company, USA. *Escherichia coli* ATCC 25922 was used as a quality control organism for the antimicrobial susceptibility test.

The plates were then incubated for 24 hrs at 37°C and the diameters of clear zones produced by antimicrobial inhibition of bacterial growth were measured to the nearest mm for each disc using a digital caliper and then classified as resistant, intermediate, or susceptible (Table 1) according to interpretive criteria of Clinical and Laboratory Standards Institute guidelines (CLSI, 2017).

Table 1. List of antimicrobial discs used, their strength, and zone diameter interpretive breakpoints in mm

Antimicrobial agent	Disc content –(µg)	Susceptible ≥mm	Intermediate mm	Resistant ≤mm
Ampicillin	30	17	15-16	14
Amoxicillin + clavulanic acid	20/10	18	14-17	13
Cefoxitin	30	18	15-17	14
ceftriaxone	30	23	20-22	19
Cephalothin	30	18	15-17	14
chloraphenicol	30	18	13-17	12
Ciprofloxcin	5	21	16-20	15
Gentamicin	10	15	13-14	12
kanamycin	30	18	14-17	13
Nalidixic acid	30	19	14-18	13
Neomycin	30	17	13-16	12
Sulfisoxazole	250	17	13-16	12
Tetracycline	30	15	12-14	11
Sulfamethoxazole +Trimethoprim	23.75/1.25	16	11-15	10

Data analysis

Data obtained from the questionnaire and laboratory activity were entered on a Microsoft Excel spreadsheet and coded appropriately. The occurrence of *Salmonella* per sample type was described as the proportion of positive samples out of total samples. Data was described using Tables.

Ethical consideration

Ethical approval was obtained from the Institutional Review Board of Aklilu Lemma Institute of Pathobiology, Addis Ababa University. Official permission was also obtained from the Addis Ababa city Agriculture Bureau. A research permit was shown to the farmers, all participants were informed about the purpose, and methods of the study, and that participation was on a voluntary basis.

Results

Of the total 151 fecal and 151 milk samples cultured for *Salmonella*, only 4 (2.7%) of the fecal samples (4/151) but none of the milk samples were positive for *Salmonella*. The distribution of *Salmonella*-positive cows among the sub-cities of Addis Ababa is shown in Table 2. The sample level prevalence of *Salmonella* among cows with different farm sizes was 3.9% (3/77), 2.0% (1/48), and 0% (0/26) for small, medium, and large farms, respectively. Prevalence was high in early lactation (5.5%) than in cows that lactated for more than 6 months (1.3%) (Table 2).

Table 2. Fecal sample level detection of *Salmonella* with respect to selected factors in dairy cows in Addis Ababa

Putative risk factors	Examined	Positive (%)
Sub cities		
Akakikality	39	0 (0.0)
Arada	20	0 (0.0)
Kirkos	30	1 (3.3)
Nifas silk	32	2 (6.3)
Yeka	30	1 (3.3)
Farming Size		
Small	77	3 (3.9)
Medium	48	1 (2.0)
Large	26	0 (0.0)
Age of the cow(years)		
<6	72	1(1.4)
6- 11	71	3 (4.2)
>11	7	0 (0.0)
Stage of lactation (Months)		
<6	55	3 (5.5)
6- 10	76	1 (1.3)
>10	20	0 (0.0)
Parity		
<3	107	3 (2.8)
>3	44	1(2.0)
Bedding		
Concrete	109	4 (3.7)
Mud	42	0 (0.0)
Udder wash		
Before milking	110	3 (2.0)
Before and After	40	1 (0.7)
No	1	0 (0.0)

Overall, in the current study, cows were sampled from 47 different farms, of which only cows from 4 farms (8.5%) were positive for *Salmonella* (Table 3).

Table 3. Farm-level prevalence of *Salmonella* among Addis Ababa dairy cows

Factors	Categories	No. of farms studied	Positive for *Salmonella* (%)
Sub cities			
	Akaki Kality	7	0 (0.0)
	Arada	12	0 (0.0)
	Kirkos	14	1 (2.1)
	Nifas Silk	6	2 (4.3)
	Yeka	8	1 (2.1)
Farm size			
	Small	33	3 (6.4)
	Medium	10	1 (2.1)
	Large	4	0 (0.0)
Bedding			
	Concrete	36	4 (8.5)
	Mud	11	0 (0.0)
Overall		47	4 (8.5)

Antimicrobial susceptibility of *Salmonella* isolates

All *Salmonella* isolates were susceptible to chloramphenicol, nalidixic acid, amikacin, gentamicin, sulfisoxazole, neomycin, and kanamycin (Table 4). One (25%) of isolates was resistant to 7 antimicrobials namely ampicillin, amoxicillin+clavulanic acid, cephalothin, ceftriaxone, sulfisoxazole, sulfamethoxazole+ trimethoprim, and, tetracycline whereas one other isolate (25%) was resistant to sulfisoxazole and tetracycline. On the other hand, the remaining 2 (50%) of the isolates were susceptible to all antimicrobials used in this study (Table 5).

Table 4. Antibiotic susceptibility profiles of *Salmonella* isolates in dairy farms in Addis Ababa

	Antibiotic Susceptibility profile		
Antimicrobials	**No. susceptible (%)**	**No. intermediate (%)**	**No. resistant (%)**
Amikacin	4 (100)	0 (0.0)	0 (0.0)
Amoxicillin+clavulanic acid	3 (75)	0 (0.0)	1 (25.0)
Ampicillin	3 (75)	0 (0.0)	1 (25.0)
Ceftriaxone	3 (75)	0 (0.0)	1 (25.0)
Cephalothin	3 (75)	0 (0.0)	1 (25.0)
Chloramphenicol	4 (100)	0 (0.0)	0 (0.0)
Ciprofloxacin	4 (100)	0 (0.0)	0 (0.0)
Gentamicin	4 (100)	0 (0.0)	0 (0.0)
Kanamycin	4 (100)	0 (0.0)	0 (0.0)
Nalidixic acid	3 (75)	1 (25.0)	0 (0.0)
Neomycin	4 (100)	0 (0.0)	0 (0.0)
Sulfisoxazole	1 (25)	1 (75.0)	2 (50.0)
Tetracycline	2 (50)	0 (0.0)	2 (50.0)
Sulfamethoxazole+ Trimethoprim	3 (75)	0 (0.0)	1 (25.0)

Table 5. Antimicrobial resistance pattern of the isolated *Salmonella* strains from dairy cows in Addis Ababa

Number of antimicrobials to which isolates were resistant	**Resistance pattern (No.)**	**No. of isolates (%)**
Zero	-	2 (50.0)
Two	G, TE (1)	1 (25.0)
seven	AMP, AMC, CF, CRO, G, SXT, TE (1)	1(25.0)

AMC = amoxicillin+clavulanic acid; AMP = ampicillin;CF= cephalothin; TE = tetracycline; CRO = ceftriaxone; G=sulfisoxazole ;SXT = sulfamethoxazole+ trimethoprim

Discussion

The current study demonstrated an overall 2.7% fecal prevalence of *Salmonella* in lactating dairy cows originating from 8.5% of the tested farms in Addis

Ababa, whereas *Salmonella* was not detected in milk samples collected from these cows. The absence of *Salmonella* from milk samples is contrary to the 3% prevalence reported previously (Addis *et al*., 2011) in the same study area. This might be due to the difference in isolation protocol employed. The current study employed confirmation of the isolates using PCR, while the previous study was based only on culture characteristics and biochemical tests. *Salmonella* is usually detected in milk due to contamination via direct or indirect contact with fecal materials during milking, storage, or transportation when there are poor hygienic practices (Holschbach and Peek, 2018). Similar to our finding in the current study, the previous study could not isolate *Salmonella* from dairy milk samples collected from central Ethiopia despite culturing several milk samples (Tigabu E. personal communication).

The overall fecal prevalence of *Salmonella* in apparently healthy lactating dairy cows is low in this study compared to previous studies in Ethiopia (Abunna *et al*., 2018; Addis *et al*., 2011) that reported 10.7% and 10.5% prevalence in Addis Ababa city and Modjo town, respectively. A study in Gondar, north Ethiopia also reported a 12.5% prevalence of *Salmonella* in lactating cows in Gondar and isolates were recovered from 44.8% of the farms investigated (Hailu *et al*., 2015). However, the current finding is in agreement with the previous report on the fecal prevalence of *Salmonella* in dairy cattle, in central Ethiopia (Eguale *et al*., 2016). The difference in isolation rate among different studies can be attributed to the difference in the study area, farm size, hygienic practices of study farms, and season of sample collection (Eguale *et al*., 2016; Pangloli *et al*., 2008). Despite low prevalence, this study shows that lactating cows are potential sources of contamination with *Salmonella* through shedding the pathogen in their feces. This could be a threat for individuals working in dairy farms and for the community that consumes unpasteurized milk and other dairy products.

The indiscriminate use of antimicrobials in the food animal production system for disease prevention and control is being implicated in the development and spread of resistance against these agents, which can easily be transferred to humans through food chains (Manyi-Loh *et al*., 2018). In this study, *Salmonella* isolates resistant to commonly used antimicrobials were recorded. One of the four isolates was multidrug-resistant to 7 of the 14 (50%) antimicrobials tested including ceftriaxone, the third-generation cephalosporin. In a previous study, none of the 30 *Salmonella* isolates from dairy farms in central Ethiopia was resistant to ceftriaxone (Eguale *et al*., 2016). As the use of third-genera-

tion cephalosporin is not common in animal production in Ethiopia, these isolates or the associated resistance genetic marker might have originated from humans, as there is close contact between humans and animals in this study area. Five (16.7%) of the isolates in the aforementioned study (Eguale *et al*., 2016) were resistant to ciprofloxacin while all of the isolates in the current study were susceptible to ciprofloxacin. On the other hand, a previous study on isolates from lactating dairy cows in Addis Ababa also showed complete susceptibility of isolates to both ceftriaxone and ciprofloxacin (Addis *et al*., 2011).

Conclusions

Despite the low level of prevalence of *Salmonella* among lactating dairy cows in this study, detection of multidrug-resistant isolates particularly an isolate resistant to ceftriaxone is a major public health concern since there is a risk of potential transmission to humans through the food chain or direct and indirect contact. Thus, appropriate measures should be instituted to protect the public and animals from infection with multidrug-resistant strains of *Salmonella.*

Acknowledgments

The authors are grateful for the farmers who collaborated in this research work. This study was supported by Addis Ababa University through Thematic Research Grant. The technical assistance of Ms. Azeb Teklu during laboratory work is highly appreciated.

Authors' contribution

HM and TE were involved in the conception of the study. HM was involved in sample collection, laboratory investigation, and preparation of the draft manuscript. HA and MG were involved in laboratory analysis. All authors read and approved the final version of the manuscript.

Funding

This study was supported through Thematic Research Grant from Addis Ababa University.

Disclosure

The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

Abunna, F., Ashenafi, D., Beyene, T., Ayana, D., Mamo, B. and Duguma, R., 2018. Isolation, identification and antimicrobial susceptibility profiles of *Salmonella* isolates from dairy farms in and around Modjo town, Ethiopia. *Ethiop. Vet. J.*, 2 (2), 92-108.

Addis, Z., Kebede, N., Worku, Z., Gezahegn, H., Yirsaw, A. and Kassa, T., 2011. Prevalence and antimicrobial resistance of *Salmonella* isolated from lactating cows and in contact humans in dairy farms of Addis Ababa: a cross-sectional study. *BMC Infect. Dis.*, 11, 222.

Bekele, B. and Ashenafi, M., 2010. Distribution of drug resistance among enterococci and *Salmonella* from poultry and cattle in Ethiopia. *Trop. Anim. Health Prod.*, 42, 857-864.

Bintsis, T., 2017. Foodborne pathogens. *AIMS Microbiol.*, l3, 529-563.

Cheng, G., Ning, J., Ahmed, S., Huang, J., Ullah, R., An, B., *et al.*, 2019. Selection and dissemination of antimicrobial resistance in Agri-food production. *Antimicrob. Resist. Infect. Control*, l8, 158.

CLSI 2018. M100: Performance standards for antimicrobial susceptibility testing: twenty-third informational supplement. (Wayne, PA).

Cohen, N.D., Neibergs, H.L., McGruder, E.D., Whitford, H.W., Behle, R.W., Ray, P.M., *et al.*, 1993. Genus-specific detection of salmonellae using the polymerase chain reaction (PCR). *J. Vet. Diagn. Invest.,* 5, 368-371.

COI 2020. Country Policy and Information Note Ethiopia: Background information, including internal relocation.

Cummings, K.J., Warnick, L.D., Elton, M., Grohn, Y.T., McDonough, P.L. and Siler, J.D., 2010. The effect of clinical outbreaks of salmonellosis on the prevalence of fecal *Salmonella* shedding among dairy cattle in New York. *Foodborne Pathog. Dis.*, 7, 815-823.

Eguale, T., Asrat, D., Alemayehu, H., Nana, I., Gebreyes, W.A., Gunn, J.S., *et al.*, 2018. Phenotypic and genotypic characterization of temporally related nontyphoidal *Salmonella* strains isolated from humans and food animals in central Ethiopia. *Zoonoses Public Health* 65, 766-776.

Eguale, T., Engidawork, E., Gebreyes, W.A., Asrat, D., Alemayehu, H., Medhin, G., *et al.*, 2016. Fecal prevalence, serotype distribution, and antimicrobial resistance of Salmonellae in dairy cattle in central Ethiopia. *BMC Microbiol.,* 16, 20.

Eng, S., Pusparjah, P., Ab Mutaliib, N., Ser, H., Chan, K. and Lee, L., 2015. *Salmonella*: A review on pathogenesis, epidemiology, and antibiotic resistance. *Fron. Life Sci.*, 8, 284-293.

Hailu, D., Gelaw, A., Molla, W., Garedew, L., Cole, L., and Johnson, R., 2015. Prevalence and antibiotic resistance patterns of *Salmonella* isolates from lactating cows and in-contact humans in dairy farms, Northwest Ethiopia. *J. Environ. Occup. Sci.,* 4, 171-178.

Holschbach, C.L. and Peek, S.F., 2018. Salmonella in Dairy Cattle. *Vet. Clin. North Am. Food Anim. Pract.,* 34, 133-154.

Keba, A., Rolon, M.L., Tamene, A., Dessie, K., Vipham, J., Kovac, J., *et al.*, 2020. Review of the prevalence of foodborne pathogens in milk and dairy products in Ethiopia. *Int. Dairy J.*, 109, 104762.

Krueger, A.L., Greene, S.A., Barzilay, E.J., Henao, O., Vugia, D., Hanna, S., *et al.*, 2014. Clinical outcomes of nalidixic acid, ceftriaxone, and multidrug-resistant nontyphoidal *Salmonella* infections compared with pan susceptible infections in FoodNet sites, 2006-2008. *Foodborne Pathog Dis.,* 11, 335-341.

Manyi-Loh, C., Mamphweli, S., Meyer, E., and Okoh, A., 2018. Antibiotic use in agriculture and its consequential resistance in environmental sources: Potential public health implications. *Molecules*, 23 (4), 795. doi: 10.3390/molecules23040795.

Minten, B., Habte, Y., Tamru, S. and Tesfaye, A., 2020. The transforming dairy sector in Ethiopia. *PLoS One,* 15, e0237456.

Pangloli, P., Dje, Y., Ahmed, O., Doane, C.A., Oliver, S.P. and Draughon, F.A., 2008. Seasonal incidence and molecular characterization of *Salmonella* from dairy cows, calves, and farm environment. *Foodborne Pathog. Dis.,* 5, 87-96.

Rahman, M.T., Sobur, M.A., Islam, M.S., Levy, S., Hossain, M.J., El Zowalaty, M.E., *et al.,* 2020. Zoonotic diseases: Etiology, impact, and control. *Microorganisms*, 8 (9), 1405. doi: 10.3390/microorganisms8091405

Varma, J.K., Molbak, K., Barrett, T.J., Beebe, J.L., Jones, T.F., Rabatsky-Ehr, T., *et al.*, 2005. Antimicrobial-resistant nontyphoidal *Salmonella* is associated with excess bloodstream infections and hospitalizations. *J. Infect. Dis.,* 191, 554-561.

WHO 2010. Isolation of *Salmonella* spp. From Food and Animal Feces. In WHO Global Foodborne Infections Network Laboratory Protocol.

WHO 2015. WHO estimates of the global burden of foodborne diseases.Foodborne diseases burden epidemiology reference group 2007-2015. In Technical Report (WHO), 1-255.