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Isolation and antimicrobial sensitivity test of *Salmonella* isolates from selected intensive dairy farms in Bishoftu town, Central Ethiopia

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Abstract
Salmonella is one of persisting foodborne pathogenic infection particularly food of animal origin including dairy cattle products. Accoringly a cross-sectional study was conducted from July to September 2021 to determine the prevalence and antibiotics resistant of *Salmonella* in Bishoftu town. To conduct the study 61 samples were collected randomly from the individual lactating cows. The *Salmonella* was recovered from the samples; conventional culture characterization of *Salmonella* was conducted by pre-enrichment on the buffered peptone water broth and enrichment in Rappaport-Vassiliadis (RV) broth, subsequently isolation was done on selective XLD and biochemical confirmation of *Salmonella* isolates. Finally, antibiotic sensitivity tests against the identified isolate were done by using eight drugs. *Salmonella* was isolated from 13/61 (21.3%, 95% CI: 11.3-31.3) of the total samples. Out of the 13 *Salmonella* isolates, 11/49 (22.4% 95% CI: 11.4-33.4) and 2/121 (16.7% 95% CI: 4.77-38.17) were isolated from milk cows and milk container respectively with no significance (P-value = 0.362). The isolates were susceptible to Ciprofloxacin, Penicillin and Vancomycin) were susceptible to the isolates, while, Streptomycin, Ampicillin, Tetracycline Rifampicillin and Cloxacillin) were resistant to the isolates. The effectiveness of such drugs like ciprofloxacin may be because they are not widely used in countries like Ethiopia and other African countries for animal's treatment. 100% rate of resistant was recorded to Ampicillin, Chloramphenicol, Cloxacillin, Streptomycin and Amoxicillin. The study indicated the prevalence of *Salmonella* with high proportion of multiple antimicrobial resistance to the commercial used in the study area. Therefore, the government, farmers and other stakeholders should give concern for dairy farm hygiene and antibiotics uses in dairy farms.

Keywords: Antimicrobial resistance, dairy farm, *Salmonella*

Introduction
Food of animal origin including milk, egg and meat are considered as the main channels of zoonotic disease transmission between the Human and Animals [11]. Salmonellosis is one of the most common foodborne bacterial diseases in the world [10, 14]. Salmonellosis is an infectious disease caused by pathogenic species of *Salmonella* which commonly isolated from food of animal origin [15]. Especially *Salmonella enteritidis* and *Salmonella typhimurium* are frequently claimed as main foodborne species of *Salmonella* species in many countries [4]. Salmonellosis is one of the most zoonotic important bacterial diseases with wit heavy economic losses through mortality and reduced milk, meat and egg production [16]. Annual estimates of 22 million cases and 200,000 deaths due to typhoid fever and 93.8 million cases of gastroenteritis and 155 000 deaths was reported due to non-typhoid *Salmonella*e [22]. The infection is most commonly reported in countries with poor standards of hygiene in food preparation and handling and where sanitary disposal of sewage is lacking [26]. *Salmonella* infections are most frequently isolated from dairy cow milk in case of poor hygiene [5]. *Salmonella* is zoonotic bacteria belongs under family of Enterobacteriaceae characterized as is a gram negative, flagellated, facultative anaerobic, rod shaped and flagellated bacterium [19, 26]. *Salmonella* is susceptibility to pH and growth temperatures (Keerthirathne *et al.*, 2016). *Salmonella* enterica serovar Dublin (S. Dublin) is multidrug resistant pathogen isolated from cattle and cattle products [9]. Antibiotics, which commonly used to treat and control salmonellosis in food animal can causes an implicated source of human infection treatment with antimicrobial resistant as human may acquire pathogenic

multi-drug resistant *Salmonella* through direct contact with livestock and livestock product such as: raw milk, cheddar cheese, and hamburger meat traced to dairy farms [20].

The public health important pathogenic and multi-drug resistant *Salmonella* is widely distributed in the raw milk in Ethiopia [14]. This bacterium is responsible for the occurrence of infectious disease called Salmonellosis due to poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest in safer equipment, and lack of public awareness toward the prevention and control of salmonellosis in developing country including Ethiopia [23]. This zoonotic bacterial disease is the major health problem of humans and animals in Ethiopia. Dairy cows and their environment harbor pathogens that pose a potential human health hazard. In Ethiopia, however, only a few studies were conducted on the risk factors for *Salmonella* contamination of cow milk and cottage cheese in Ethiopia [7, 14]. To recommend the community is risk area we have to ensure the presence and determine the prevalence and resistance status to the commonly used veterinary antibiotics against the *Salmonella* is a key strategy to aware the community to control and prevent the salmonellosis in area of veterinary and human medicine. Therefore, the current study was to determine the prevalence and antibiotics resistant of *Salmonella* in Bishoftu town. The information generated through such study have great role to control and prevent zoonotic disease as it alerts the livestock policy makers who works with community with low information about the zoonotic bacterial infection including non-typhoidal *Salmonella* and their developments antimicrobial resistant to antibiotics commonly used in areas of Veterinary medicine and human medicines.

Materials and Methods

Study design

A cross-sectional study on the Prevalence and antimicrobial resistant was conducted from April to September 2021 on lactating dairy cows in two selected farms of CVMA, AAU farms and Ethiopian Meat and dairy industry development institute (ENIDI) in Bishoftu town. Accordingly, 61 samples were collected randomly from the individual lactating cows. Milks were collected with 50 ml falcon tube, while swab sample of containers were collected in 10 ml test tube filled with transport media (buffered peptone water). Then all samples were handled and transported to AAU, CVMA, department of MIVP with Ice box. The *Salmonella* was recovered with First the pre-enrichment buffered peptone water broth, followed by enrichment in selective broth, of Rappaport- Vassiliadis (RV) broth and the subsequent isolation is done on selective XLD and biochemical confirmation of *Salmonella* isolates by using different biochemical tests that included TSI agar, Simmon's citrate agar, indole and MR-VP tests. Finally, antibiotic sensitivity tests against the identified isolate were done by using eight drugs.

Laboratory procedure

Each milk samples collected from the study areas were pre-enriched in buffered peptone water (1:9 ml) ratio while milk containers were as it collected in 9 ml of buffered peptone water and incubated at 37 °C for 24 hrs. At the second day, after 24 hrs. Incubation, 0.1 ml of the pre-enriched sample was transferred into a tube containing 10 ml of Rappaport-Vassiliadis medium (RV broth) and incubated at 41.5 °C for 24 hours. Then, a loop full of inoculums from RV were cultured on the Xylose lysine deoxycholate (XLD) agar and incubate at 37 °C for 24 for *Salmonella* identification by characterizing of a black center and a lightly transparent zone of reddish color. Then, according to the standard (WHO, 2010), biochemical test including: triple sugar iron (OXOID CM0277, England) agar, Simmon's citrate agar (HIMEDIA M099, India), (HIMEDIA M111A, India), Methyl red, -Voges-Proskauer (HIMEDIA M070, India) broth and indole test were done on colony picked from nutrient agar after transferring of all suspected *Salmonella* colonies onto nutrient agar and incubated for 24 to 48 hrs.' at 37 °C. the sample were interpreted as *Salmonella* if it was Methyl red positive, indole negative, citrate negative, Hydrogen sulfide positive, no change on alkaline butt, change on acid butt (H_2S), VP positive and indole negative. Finally, according to the National Committee for Clinical Laboratory Standards (NCCLS, 2012), The resistant of the isolates were tested with their respective concentration (ampicillin, streptomycin, ciprofloxacin, Vancomycin, tetracycline, penicillin, Cloxacillin, and Pivampicillin. Biochemically confirmed well- isolated colonies were grown on nutrient agar and transferred into tubes containing 5 ml of Tryptone soya broth (Oxoid, England) and incubated at 37 ºC for 24 hrs. Until it achieved the 0.5 McFarland turbidity standards. Then the broth culture was spread on muller Hinton agar with cotton swab, then the discs of the selected antibiotics were putted on the put on the plate and incubated at 37 ºC for 24 hr. Finally, the disc diameter was measured with ruler and mid-point were determined.

Data analysis

The raw data of each sample were arranged, organized, coded and entered to Microsoft office excel 2016. Then the prevalence of the data was analyzed through descriptive analysis with percentage. The results of analyses were mostly described in proportion. Proportional was estimated as the numbers of samples detected positive to *Salmonella* from the total sample tested as well as the numbers of antimicrobial resistant isolate to the detected positive isolate. The statistical significance of the prevalence of *Salmonella* between the milk sample and milk containers was determined on R Studio by using chi-square analysis in which comparation with P-Value less five ($P\text{-Value} < 0.05$) was decided as statistically significant. The resistant of the isolates to selected antibiotics was determined by mid-point of each isolate against all selected antibiotics discs inhibition on the 2016 excel.

Results and Discussion

Enteric pathogens such as *Salmonella* Enterica cause morbidity and mortality have diverse group of pathogens that have evolved to survive in a wide range environment and across multiple hosts [12]. Consequently, economic losses may be resulted from mortality and poor growth of infected animals as well as the risk of transmission to humans either through food chain or direct animal contact. Hence, detection is essential to control *Salmonella* on farm and its spread to the public [1]. *Salmonella* species are easily transmitted to human through poor hygiene of food of animal origin either at farm or processing plants [2]. The raw milk quality can easily depreciates more frequently in the developing countries including Ethiopia due to technical and

economic conditions being unfavorable than developed countries [11]. The temperature of the water used in dairy farm for cleaning of cow's udder, combining of milk of different cows, milk containers, refrigerator and milk filtration were identified as risk factor for the prevalence of *Salmonella* inidairy cow milk [7]. The current cross-sectional study was designed to isolate, identify and to determine the antimicrobial susceptibility of *Salmonella* among lactating dairy cows in two selected farms of CVMA, AAU farms and Ethiopian Meat and dairy industry development institute in Bishoftu town.

Salmonella was isolated from 13/61 (21.3%, 95% CI: 11.3-31.3) of the total samples. Out of the 13 *Salmonella* isolates, 11/49 (22.4% 95% CI: 11.4-33.4) and 2/121 (16.7% 95% CI: 4.77-38.17) were isolated from milk cows and milk container respectively with no significance (P-value = 0.362) difference between sample sources (Table 1).

Table 1: Isolation rate of *Salmonella* from different samples

Sample Type	No of observe	Positive	Prevalence (%)	Chi-sq	P-Value
Milk	49	11	22.4 (CI:11.4-33.4)	0.83	0.362
Milk Cont.	12	2	16.7 (CI: 4.77-38.17)		
Total	61	13	21.3 (CI:11.3-31.3)		

The result obtained from the milk was in agreement with that of the 20% reported by Tadesse and Dabassa (2012) in Ethiopia, but it was lower when comparing the 29.4% prevalence of *Salmonella* in milk reported by Cao *et al.*, [9] in his study on the Prevalence of *Salmonella* and Antimicrobial Resistance in Isolates from Food Animals. The result of milk container was higher than that 10% (1/10) of *Salmonella* isolates from bulk tank milk (Abrar *et al.*, 2020). But it was lower than the report of 19% (4/21) [3] in milk samples contaminated with *Salmonella* spp. Milk containers can contaminated from milking environmental sites and animal sites [21]. Also the current prevalence of *Salmonella* was great difference with that of Azooz *et al.*, [6] who reported the overall prevalence of 13.3% Typhimurium from dairy herd. The current study was relatively close to the prevalence of *Salmonella* reported by [7] 19.7% at the production and 21.3% at milk collection centers. The prevalence of *Salmonella* isolated from raw milk and milk containers swab were 22.4% and 15.3% respectively. The overall prevalence of raw milk *Salmonella* in current study was greater than the report of Asefa *et al.*, [5] that shown the prevalence of 9.3% (14/151) [25].

The results obtained in the study indicated that there was high prevalence of *Salmonella* in the farm of Addis Ababa University, College of Veterinary Medicine and Agriculture (20%) when it was compared with that of Ethiopian meat and dairy industry institute (Table 2).

Table 2: Occurrence of *Salmonella* between the farms

Farms	No of Observe	Positive	Prevalence	Chi-sq	P-Value
CVMA	22	6	27.3% (CI: 8.7-45.9)	2.498	0.114
EMDIDI	39	7	17.9%(CI:5.8-29.98)		
Total	61	13	21.3%(CI:11.3-31.3)		

CVMA: College of Veterinary Medicine and Agriculture
EMDIDI: Ethiopian meat and dairy industry development institute

The results obtained in the study indicated that there was high prevalence of the salmonellosis in the farm of Addis Ababa university, college of veterinary medicine and agriculture when it was compared with that of Ethiopian meat and dairy industry institute (4/20), (20%) and 5/35 (14.2%), respectively). Hygienic and management practice, stocking density, type and amounts of feed, accessible water supplies, usage of contaminated utensils, housing type, ventilation, and movement of animals, calving environment, and production facilities are the main environmental risk factors of *Salmonella* in dairy farms [17]. These variations may be explained by the differences in location, hygienic practices and the availability of potable water. The quality of agricultural practices observed on the farms may also cause variations in levels of contamination with Antimicrobial resistant *Salmonella* were increasing due to the use of antimicrobial agents in food animals at the sub-therapeutic level or prophylactic doses, which might promote on-farm selection of antimicrobial-resistant strains and markedly increase the human health risks associated with consumption of contaminated meat products [25]. The pathogen can be shed in feces, milk, and colostrum and persist in asymptomatic cattle, leading to spread and outbreaks in herds [13]. In this study, the isolates were susceptible to Ciprofloxacin, Penicillin and Vancomycin) were susceptible to the isolates, while, Streptomycin, Ampicillin, Tetracycline Rifampicillin and Cloxacillin) were resistant to the isolates. The effectiveness of such drugs like ciprofloxacin may be because they are not widely used in countries like Ethiopia and other African countries for animal's treatment [3]. Ampicillin, Chloramphenicol, Cloxacillin were resistance in the rate of (100%) to Streptomycin and Amoxicillin [24].

Table 3: Antimicrobial sensitivity test results

No	Antibiotics	MPI	Status
1	Ciprofloxacin	30.8	Sensitive
2	Streptomycin	10.23	Resistant
3	Penicillin	19	Sensitive
4	Ampicillin	11	Resistant
5	Vancomycin	13.16	Sensitive
6	Tetracycline	11	Resistant
7	Rifampicillin	11	Resistant
8	Cloxacillin	10	Resistant

MPI: Mean of the Percent of Inhibition

Fecal contamination and cross contamination from container and milk handler hand in case of poor milking hygiene is a key risk factor for *Salmonella* to contaminating bulk cow milk in dairy farms [6]. The possible for horizontal transmission of resistance characters and other virulence factors or plasmids from *Salmonella* to other microbes, including within the human gut, may exist. Resistance traits in *Salmonella* can be genetically developed and transferred to another Enterobacteriaceae. The degree of all these is not known in the dominant conditions in Ethiopia since undiagnostic and under diagnosis can be communal and prescriptions are virtually empirical. Large-scale intensive farming combined with use of antibiotics (which is empirical in most developing countries) in animals is likely to increase in the coming decades, thereby endorsing on-farm selection of antibiotic-resistant strains and markedly increasing the human health risks [26].

Conclusion and Recommendations

The isolation of 21.3% *Salmonella* at dairy farms level

showed that dairy cattle and their environment are important sources of milk contamination. Detection of high proportion of multiple antimicrobial resistant isolates in the dairy farms' alerts concern for animal and public health as these drugs are used widely for treatment and prophylaxis of various bacterial infections in animals and humans. The prevalence of *Salmonella* in our study area indicated that there was high prevalence of *Salmonella* in the study area when comparing with the previous study conducted in Ethiopia. Therefore, based on the above conclusion, the following recommendations were forwarded:

- Develop good hygiene status of the dairy farms in order decrease cross contamination of *Salmonella* from milking containers and cattle's environment.
- Attention should be given in selection and dose of antimicrobials used in animals and human treatments.
- Training should be given for both farmers and Milk consumer on the Prevalence, risk factor and antibiotics uses.
- Further study to identify the source of contamination, risk factor and molecular characterization of *Salmonella* and antibiotic resistance test should be done.

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