Isolation, identification and antimicrobial susceptibility profiles of *Salmonella* isolates from dairy farms in and around Modjo town, Ethiopia

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Abstract

Foodborne bacterial diseases are a serious challenge to human and animal health. *Salmonella* is a zoonotic foodborne pathogen and the etiologic agent of salmonellosis. A cross-sectional study was conducted from January 2016 to April 2016 on small scale and large scale dairy farms in and around Modjo town, Ethiopia. The main objectives of the study were to isolate and identify *Salmonella* from lactating cows, personnel's' and equipment at farms and to determine the *in vitro* antimicrobial resistance profiles of the isolates. A total of 266 samples consisting of fresh cow milk, fecal sample, pooled milkers' hand swab, pooled bucket swab, tank swab, and tank milk were collected from 21 dairy farms (n=20 smallholders, n=1 large scale farm). The samples were examined for the presence of *Salmonella* following standard techniques and procedures outlined by the International Organization for Standardization. Kibry-Bauer disk diffusion test was used for the antimicrobial susceptibility testing. *Salmonella* was isolated from 28/266 (10.5%) of the total samples. Out of the 28 *Salmonella* isolates, 18 (64.3%), 3 (10.7%) and 7(25%) were from lactating cows, personnel's', and equipment, respectively. Out of the 28 isolates subjected to antimicrobial susceptibility testing, all isolates were resistant to at least one or more antimicrobials tested. Accordingly, 96.4% (27/28), 82.1% (23/28) and 75.0% (21/28) isolates were resistant to tetracycline, kanamycin and nalidixic acid, respectively. Multiple drug resistance (resistance to two or more antimicrobials) was detected in 27(96.4%) of the isolates. Multiple antimicrobial resistance was observed in 100% (18/18), 7.4% (2/23) and 100% (7/7) of isolates obtained from lactating cows, personnels', and equipment, respectively. High proportion of multiple antimicrobial resistant isolates (96.4%) in

the dairy farms alerts concern for animal and public health as these drugs is used widely for treatment and prophylaxis in animals and humans.

Keywords: Antimicrobials; Dairy farms; Isolation; Modjo; Multidrug resistance; *Salmonella*

Introduction

Foodborne diseases are a public health problem in developed and developing countries. More than 250 different foodborne diseases have been described. Most of these diseases are infections caused by a variety of bacteria, viruses and parasites. Other diseases are poisonings, caused by harmful toxins or chemicals like poisonous mushrooms (CDC, 2005). Bacteria that cause foodborne diseases include *Salmonella, Campylobacter, Listeria,* pathogenic *Escherichia coli, Yersinia, Shigella* and *Enterobacter*. Foodborne bacterial diseases are a serious challenge to human and animal health. *Salmonella* is a zoonotic foodborne pathogen. Salmonellosis is a major bacterial enteric illness in both humans and animals and it is the most common foodborne bacterial disease worldwide (Forshell and Wierup, 2006).

Salmonella species belong to Gram negative, rod shaped, facultative intracellular bacteria that successfully infects a wide variety of hosts. *Salmonella* is comprised of two species, *Salmonella bongori* and *Salmonella enterica* (Guibourdenche *et al*., 2010). Based on the bacterial outer membrane surface 'O' antigen, and flagellar 'H' antigen more than 2,700 different serovars of *Salmonella* has been characterized (Collazo and Galan 1997). Out of these 2,700 serovars, nearly 1500 belong to the *Salmonella enterica* subsp. enterica. Serovars of the enterica sub species can be divided into three groups depending upon their ability to infect a wide variety of hosts: Serovars which have a broad host range also called as unrestricted serovars as these infect nearly all animals and pose a greater zoonotic potential than their other counterparts (*Salmonella* Typhimurium and *Salmonella* Enteritidis) (Bäumler *et al.*, 1998), Serovars which accidentally infect hosts other than their most adapted or preferred (*Salmonella* Dublin and *Salmonella* Choleraesuis) (McCuddin *et al*., 2006), and serovars which are restricted to one specific host only (*Salmonella* Typhi and *Salmonella* Gallinarum) (Uzzau *et al.*, 2001).

Salmonella is transmitted to animals and humans through the fecal-oral route. Animals can become infected after ingestion of feed and water contaminated with *Salmonella*. Similarly, humans can become infected by foodborne transmission or after direct or indirect contact with infected animals (Hoelzer *et al*., 2011), consumption of contaminated food products (milk, eggs, and meats), cross contamination through direct contact of foods to contaminated surfaces such as stainless steel, hanging material, knife, bucket where milk are collected are a key mechanism for pathogens to contaminate food products (Kusumaningrum *et al*., 2003; Plym and Wierup, 2006). In livestock, clinical signs, typically appear 6–24 h after exposure and include profuse diarrhea, fever, dehydration, in appetence, foul-smelling feces, and mucus or blood in feces (Cummings *et al*., 2010). Disease manifestations in people include diarrhea, fever, abdominal cramps and septicemia in severe cases, appearing 12–72 h after ingestion. *Salmonella* can also be subclinical in both humans and animals (Murase *et al*., 2000).

Despite the controls that have already been put into place, *Salmonella* infection arising from contaminated food continues to be an immense problem with millions of cases occurring annually throughout the world. In addition to the misery caused, financial loss is enormous (Hendriksen, 2003). Salmonellosis is a costly disease to dairy producers on account of mortality, treatment expenses, reduced milk yield, and weight loss within the herd. Infected cattle can be either clinical or subclinical, shedding *Salmonella* in their faeces. Thus dairy producers need to be aware that *Salmonella* can be found on their farms within apparently healthy cows, which is important in terms of food safety risks (Callaway *et al*., 2005). Using antimicrobial agents for cattle have been implicated as a source of human infection with antimicrobial resistant (AMR) *Salmonella* through direct contact with livestock and consumption of raw milk, meat and contaminated materials (Alexander *et al*., 2009). Antimicrobial resistant *Salmonella* are increasing due to the use of antimicrobial agents in food animals at subtherapeutic level or prophylactic doses for growth promotion and markedly increase the human health risks associated with consumption of contaminated milk and meat products (Endrias Zewdu and Cornelius 2009), through mutation, acquisition of resistance encoding genes (Fluit, 2005) and irrational use of antimicrobials in food animals (Fluit, 2005; Takele Beyene and Berihun Tesega, 2014).

Different studies conducted in Ethiopia revealed fragmented substantial prevalence as well as antimicrobial susceptibility of *Salmonella* in veterinary and public health setups (Daniel Alemayehu *et al.,* 2003; Bayleyegn Molla *et al.,* 2003; Getahun Ejeta *et al.*, 2004; Wassie Molla *et al.*, 2006; Endrias Zewdu and Cornelius, (2009); Zelalem Addis *et al.,* 2011; Deresse Hailu *et al.*, 2015; Takele Beyene *et al.*, 2016). However, reports from coinciding study on apparently healthy animals at farm level, personnel and equipment used in the farms is limited especially in the current study area. The screening of milk and other dairy products for pathogenic organisms will play a vital role in curtailing human infection. Investigation of the prevalence and antimicrobial resistance of *Salmonella* from cattle and in contact human in dairy farms is of paramount importance to design methods to minimize the possible transmission of *Salmonella* between humans and cattle. Moreover, it is also important in combating the emergence of antibiotic resistant strains of *Salmonella* (Zelalem Addis *et al.*, 2011). Therefore, the aims of the current study were to isolate *Salmonella* from dairy cows, personnel hand and equipment and evaluate the antibiogram pattern of the isolates in selected dairy farms in and around Modjo town.

Materials and Methods

Study area

The study was conducted in and around Modjo town from January 2016 to April 2016. Modjo is the administrative center of Lome district, located in the East Shewa Zone of the Oromia Region, Ethiopia. It is located at 66 Km Southeast of Addis Ababa and lies at latitude 8°35'N and longitude 39°7'E at an altitude of 1790 meters above sea level. The area gain rainfall twice a year those known as long and short rainy season. The main rainy season extends from June to September. The average annual rainfall, temperature, and mean relative humidity are: 776mm, 19.4 ºC and 59.9% respectively (CSA, 2005).

Study population

The study population is lactating dairy cows in Modjo town and the study animals were apparently healthy dairy cows in small and large scale dairy farms located in and around Modjo town. The farms were selected by using simple random sampling strategies based on data obtained from the Lome district livestock and fishery resource development. The study populations were divided according to their location as urban (Modjo town) and peri-urban (the sur-

rounding area within Lome district). In this study, the majority of farms found in the study area were small scale having herd sizes not more than six cows. All of the available lactating cows present in each farm were sampled except the one large scale dairy farm in which cows were sampled by simple random sampling. According to personal observation, the hygienic status of the cows and their environment was more or less good even though some animals were reared under poor hygienic condition plus in a manner mixed with other activities of the households. Farm equipment used in the milking and storage of milk and personnel's (milkers) were also part of the study.

Study design

A cross-sectional study was carried out to isolate, identify and detect antimicrobial susceptibility profile of the *Salmonella* from dairy farms. Sampling days were randomly assigned and each farm was visited only once during the study period. Types of sample collected include udder milk, tank milk, pooled milkers' hand swab, pooled buckets swab and tank swab. Prior to sample collection, cooperation letter was sent to Lome district livestock and fishery resource development bureau and animal health technician was assigned and sampling in each dairy farm was undertaken in collaboration with them.

Sampling and sample size determination

The farms were randomly selected for this study based on the availability and accessibility of study animals. All animals fulfilling the inclusion criteria (apparently health animals) were considered. The minimum sample size was calculated by using the formula given by Thrusfield (2007): $n = Z^2 p (1-p)/ d2$ where n = sample size, p (expected prevalence) = 28.6% (Zelalem Addis *et al*., 2011), d (absolute precision) = 0.05 at 95% confidence interval. Accordingly, the sample size was calculated to be 314. However, in this study, a total of 266 samples were collected (because of unwillingness of the owners, inaccessibility of farms and limited resources) from twenty small scales (less than 20 dairy cows) and one large scale (more than 20 dairy cows) selected dairy farms for all sample types.

Sample collection and transportation

Samples from dairy cows (milk and faeces), hands of personnel working in the farms (milkers) and from equipment were aseptically collected from the se-

lected dairy farms. Samples from dairy cows were collected from apparently health lactating cows. Fresh faecal samples were collected directly from the rectum of healthy lactating dairy cows using disposable gloves in to sterile plastic bags. Milk samples were collected after the teats were scrubbed vigorously with a pledge of cotton moistened with 70% ethyl alcohol and the first 3-4 streams of milk were discarded. The nearest teats were sampled first, then toward far ones. The collecting vial was held as near horizontal as possible and by turning the teat to a near horizontal position. Approximately 10 ml of milk was collected aseptically from all teats in a sterile test tube. Pooled milkers' hand swab, tank swab, and pooled buckets swab were collected before the beginning of milking process by using a sterile cotton swab.

Samples were properly coded based on collection date, sample source and sample type. Source of sample was classified as animal, personnel and equipment. Types of samples collected in quantity were udder milk (91), faeces (91), pooled milkers' hand swab (21), pooled buckets swab (21), tank swab (21) and tank milk (21). A total of 266 samples were collected from animals (n=182), personnel (n=21), and equipment (n=63). Samples were collected early in the morning around 6:00 to 7:00 AM and in the afternoon around 4:00 to 6:00 PM by arranging time in communication with the milkers' and owners of the farms. Then samples were immediately transported under cold condition (ice box) to the Microbiology Laboratory of College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, which is about 20 km from sampling area for culturing. Upon arrival, samples were processed separately by pre-enriching in pre-enrichment media or were stored overnight in a refrigerator at +4^{o}C until examined the next day. Then further processes were followed after samples were incubated for 24 hrs.

Isolation and identification of *Salmonella*

The isolation and identification of *Salmonella* was performed at the Microbiology laboratory of College of Veterinary Medicine and Agriculture, Addis Ababa University by using techniques recommended by International Organizations for Standardization (ISO-6579, 2002), and those recommended by the World Health organization (WHO) Global foodborne infections network (formerly WHO global *Salmonella* Surveillance) (WHO, 2010; Hendriksen, 2003). The detection of *Salmonella* necessitates four successive stages: Upon arrival or thawed refrigerated samples were processed separately and the appropriate size of processed samples (25g) are incubated within 225ml of Buffered pep-

tone water for pre-enrichment purposes (1:9)(CONDA Cat,1402.00, England) at 37±1ºC for 18±2hrs. Tetrathionate Broth (TM MEDIA, India) and Rappaport Vassiliadis *Salmonella* Enrichment Broth (HIMEDIA, India) were used for selective enrichment of all samples whereas Xylose lysine Desoxycholate agar (OXOID CM0469, England) and *Salmonella-Shigella* (SS) agar (OXOID CM0099, England) plates were used for plating out and identification purpose. For confirmation, five presumptive *Salmonella* colonies (or less depending on availability) were selected from every selective plating media. The selected colonies were streaked onto the surface of pre-dried Nutrient agar (OXOID CM0003, England) plates in a manner that allow isolated colonies to develop and incubated at 37±1ºC for 24±3hrs for further confirmation with biochemical tests. All suspected *Salmonella* colonies were picked from the nutrient agar and inoculated into the following biochemical tubes for identification: tryptone soya broth (OXOID CM0129, England), triple sugar iron (OXOID CM0277, England) agar, Simmon's citrate agar (HIMEDIA M099, India), urea broth (HIMEDIA M111A, India), Methyl red-Voges-Proskauer (HIMEDIA M070, India) broth and then incubated for 24 to 48 hrs at 37ºC.

Antimicrobial susceptibility test

The antibiotic susceptibility tests of the *Salmonella* isolates were performed according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2006) by using Kibry-Bauer disk diffusion test on Muller-Hinton agar (OXOID CM0337, England). Pure colonies on nutrient agar were taken with a wire loop and transferred to a tube containing 5 ml of saline water and emulsified. The broth culture was incubated at 37°C for 4 hrs until it achieved the 0.5 McFarland turbidity standards. Sterile cotton swab was dipped into the suspension and the bacteria were swabbed uniformly over the surface of Muller- Hinton agar plate within a sterile safety cabinet. The plates were held at room temperature for 15 minutes to allow drying. Antibiotic discs with known concentration of antimicrobials were placed and the plates were incubated for 24 hrs at 37°C.

Each isolate was tested for a series of eleven antimicrobials. amoxicillin (AML) (25 µg), ampicillin (AMP) (10 µg), cefoxitin (FOX) (30 µg), chloramphenicol (C) (30 µg), gentamycin (CN) (10 µg), streptomycin (S) (10 µg), kanamycin (K) (30 µg), nalidixic acid (NA) (30 µg), ciprofloxacin (CIP) (5 µg), tetracycline (TE) (30 µg) and trimethoprim-sulphamethaxazole (SXT) (25 µg), all from Oxoid com-

pany, England. Following incubation, the diameters of clear zones produced by antimicrobial inhibition of bacterial growth were measured to the nearest mm for each disc using transparent straight line ruler and then classified as resistant, intermediate, or susceptible according to published interpretive chart of CLSI.

Data management and analysis

Data was analyzed by using Stata version 12 (2011, College Station, TX: StataCorp LP, USA). Descriptive analysis was used to describe the result of proportion analysis. Proportion was estimated as the number of samples detected positive to *Salmonella* isolation from the total sample analyzed. Chi-square test was done to study association between *Salmonella* isolates and variables considered (sampling area, sample source, sample type and herd size). The significance level was set at 0.05.

Results

Salmonella was isolated from 28/266 (10.5%) of the total samples. Out of the 28 *Salmonella* isolates, 18 (64.3%), 3 (10.7%), (and 7(25%) were from lactating cows, personnel's', and equipment, respectively.

From a total of 21 dairy farms included in the current study, *Salmonella* was isolated from 17 (80.95%) farms. From a total of 91 lactating cows examined, 17.6% (16/91) were positive for *Salmonella* either from milk or faecal samples or both. From the cows confirmed *Salmonella* positive, 68.75% (11/16) were positive from milk sample and 43.75% (7/16) were positive from faecal sample. Two cows were positive both from the faeces and milk sample. There is no statistically significant difference between isolates derived from milk and faecal samples of studied dairy cows ($\chi 2=3.709$, p=0. 592) (Table 1).

Table 1. Distribution of *Salmonella* isolates in dairy farms

Sample types	Number of samples	
	Examined	Positive (%)
Udder milk	91	11 (12.1)
Fecal sample	91	7 (7.7)
Hand swab	21	3 (14.3)
Bucket swab	21	2 (9.5)
Tank swab	21	1 (4.8)
Tank milk	21	4 (19.0)
Total	266	28 (10.5)

All the 28 isolates were tested against eleven commonly used antimicrobials. All isolates were resistant at least to one or more antimicrobials. Twenty seven of the 28 isolates were resistant to two or more antimicrobials. The antibiotic susceptibility profiles of the isolates showed that the isolates were 96.4%, 82.1% and 75.0% resistant to tetracycline, kanamycin and nalidixic acid, respectively. On the other hand, all isolates were 100% sensitive to gentamicin and ciprofloxacin (Table 2).

Table 2. Antibiotic Susceptibility Profiles of *Salmonella* isolates in dairy farms

Antimicrobials	**Antibiotic Susceptibility profile**		
	No.sensitive (%)	**No. intermediate (%)**	**No. resistant (%)**
Kanamycin	2 (7.1)	3 (10.7)	23 (82.1)
Nalidixic acid	0 (0.00)	7 (25.0)	21 (75.0)
Gentamicin	28 (100.0)	0 (0.00)	0 (0.00)
Cefoxitin	25 (89.3)	0 (0.00)	3 (10.7)
Streptomycin	16 (57.1)	9 (32.1)	3 (10.7)
Chloramphenicol	14 (50.0)	9 (32.1)	5 (17.9)
Tetracycline	0 (0.00)	1 (3.6)	27 (96.4)
Amoxicillin	10 (35.7)	11 (39.3)	7 (25.0)
Ampicillin	17 (60.7)	0 (0.00)	11 (39.3)
Ciprofloxacin	28 (100)	0 (0.00)	0 (0)
Trimethoprim-Sulfamethoxazole	22 (78.6)	3 (10.7)	3 (10.7)

Multiple antimicrobial resistances(resistance to two or more antimicrobials) was detected in 96.4% (27/28) of the isolates. A total of seven different antimicrobial resistance patterns were observed (Table 3).

Table 3. Antimicrobial resistance patterns of *Salmonella* isolates

Number of antimicrobial resistance	Antimicrobial resistance patterns (number of isolates)	Number of isolates (%)
Two	NA, TE (3) K, TE (2)	5(18.5)
Three	K, NA, TE (7) K, TE, AMP (1)	8(29.6)
Four	K, NA, TE, AMP (2) C, TE, AMP,(1) NA, TE, AML,AMP (1) K, NA, TE, SXT (1) K, TE, AML, SXT (1) K, NA, S, TE (1)	7(25.9)
Five	K,NA, C, TE, AMP (1) K, NA, TE, AML, AMP (2) K, NA, FOX, C, TE (1)	4 (14.8)
Six	K, NA, FOX, S, AML,AMP (1)	1 (3.7)
Seven	K, NA,C,TE,AML,AMP,SXT (1)	1 (3.7)
Eight	K, NA, FOX, S, C, TE, AML, AMP (1)	1 (3.7)

Key: K (Kanamycin), NA (Nalidixic acid), CN (Gentamicin), FOX (Cefoxitin), S (Streptomycin), C (Chloramphenicol), TE (Tetracycline), AML (Amoxicillin), AMP (ampicillin), CIP (Ciprofloxacin), SXT (Trimethoprim-sulfamethoxazole).

From total isolates derived from all three sources, 96.4% of the isolates were resistant to two or more of the antimicrobials tested. The isolates from milk samples were 100% resistant to tetracycline and 81.8% resistant to nalidixic acid and kanamycin. On the other hand, all isolates from feces were 100% resistant to tetracycline and 85.7% of the isolates were resistant to kanamycin and nalidixic acid.

Discussion

Salmonella infection in dairy cattle persists to be a major problem worldwide. Considerable economic losses were manifested through 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 of animals contacting humans and equipment are essential to control *Salmonella* on-farm and its spread to the public (Plym and Wierup, 2006; Rotimi *et al.*, 2008). The

proportion of *Salmonella* isolated in this study (10.5%) is lower than 20% in raw milk from Kersa district; Ethiopia (Teshome Tadesse and Anbessa Dabassa, 2012). This difference may be attributed to the difference in the source of sample. But, it is consistent with 10.76% in lactating cows and in contact humans in dairy farms of Addis Ababa (Zelalem Addis *et al.*, 2011).

In this study the prevalence of *Salmonella* in apparently healthy lactating dairy cows (milk and faecal samples) (17.6%) is higher than a similar report in Gondar town (12.5%) (Deresse Hailu *et al.*, 2015). Hence lactating cows could be potential sources of *Salmonella* infection for individuals working in dairy farms and for the community at large. Fecal prevalence of *Salmonella* among lactating dairy cattle in the current study was 7.7% (7/91) which is interestingly similar with the fecal Salmonella isolation rate of 7.7% in lactating cows and in contact humans in dairy farms of Addis Ababa (Zelalem Addis *et al*., 2011) and 7.3% in dairy cows in USA (Blau *et al.,* 2005). However, it is lower than the fecal *Salmonella* isolation rate of 9.7% in United States (Callaway *et al*., 2005). This study also disagrees with a report of 1.56% prevalence in Egypt on fecal shedding of Salmonella among dairy cattle (Mohamed *et al.*, 2011). The current study also revealed 14.3% of Salmonella isolates from pooled milkers' hand swab which is lower than that reported by Takele Beyene *et al*. 2016 (28.6%) from Asella Municipal abattoir but higher than the work of Akafete Teklu and Haileleul Negussie (2011) 8.9% in slaughtered small ruminants and environment in Modjo export abattoir.

The difference in amount and relative occurrence of *Salmonella* isolate between the present and previous studies at different areas of the Ethiopia could be attributed to difference in risk factors that contribute to the occurrence of *Salmonella*. These are host related risk factors that include age, breed, the physiological state of the animals, feeding strategies, vaccination status (Liza, 2003). Environment related risk factors such as hygienic and management practice, stocking density, type and amounts of feed, accessible water supplies, usage of contaminated utensils, housing type, ventilation, movement of animals, calving environment, and production facilities in different areas also play role for *Salmonella* occurrence (Karin *et al.*, 2011).

Antimicrobial use in animal production systems has long been suspected to be a cause of the emergence and dissemination of antimicrobial resistant *Salmonella*. Improper use of antimicrobials in both human and veterinary medicine

has contributed to development and dissemination of antimicrobial resistant pathogens (Zelalem Addis *et al*., 2011; Tajbakhsh *et al.*, 2012). In this study, resistance to two or more of antimicrobials (96.4%) was observed. This is higher than studies conducted in Ethiopia (Zelalem Addis *et al.*, 2011; Endrias Zewdu and Cornelius, 2009; Abebe Mekuria *et al*., 2014; Teshome Tadesse and Anbessa Dabassa, 2012; Anbessa Dabassa and Ketema Bacha, 2012; Fadlalla *et al* (2012) from Sudan, Stevens *et al.*(2006) from Senegal, and Lagos, Nigeria (Stella *et al.*, 2009). This difference may be due to the increasing rate of inappropriate utilization of antimicrobials in the dairy farms which favors selection pressure that increased the advantage of maintaining strains of bacteria carrying resistance genes (McGeer *et al.*, 1998; Mathew *et al.*, 2006). A study in Alexandria Egypt (Mohamed *et al.*, 2011) reported that 85.7% of *Salmonella* species isolated from dairy cattle were sensitive to ampicillin and tetracycline. This result strongly disagrees with the current study in which 96.4% and 39% of the isolates were resistant to tetracycline and ampicillin, respectively. Resistance rates to ampicillin and tetracycline is very high when compared to results documented in America (Blau *et al.*, 2005) reported as 4.4 % and 12.2 % resistance levels, respectively.

This finding is in line with a report in Sudan (Fadlalla *et al*., 2012) in which Salmonella isolates from human and cattle were 100% susceptible to ciprofloxacin. The high sensitivity rate observed among Salmonella isolates in the current study to gentamicin and ciprofloxacin (100%) which is higher than 73.3% and 83.3% reported by Zelalem Addis *et al.* 2011 and 75% and 95% reported by Teshome Tadesse and Anbessa Dabassa, 2012; for both antimicrobial agents, respectively might be due to difference in sample size, presence of different strain of the bacteria, difference in frequency and dosage of drugs used.

A single isolate from fecal sample was MDR to 8 antimicrobials namely kanamycin, nalidixic acid, cefoxitin, streptomycin, chloramphenicol, tetracycline, ampicillin, and amoxicillin followed by an isolate from udder milk resistant to seven antimicrobials: kanamycin, nalidixic acid, chloramphenicol, tetracycline, ampicillin, amoxicillin, and trimethoprim-sulphamethaxazole. The possible reason for high rate of AMR level of *Salmonella* might be due to the increasing rate of irrational use of antimicrobials in the dairy farms, frequent usage both in livestock and public health, use of counterfeit drugs in animal husbandry (Guthrie, 1992), self-medication due to easy access to antimicrobials without prescription in public health sector and administration of subtherapeutic dose

of antimicrobials to livestock for prophylactic or nutritional purpose in food animals (Acha and Szyfers, 2001; Tadesse Birhanu *et al.*, 2014).

Antimicrobial-resistant *Salmonella* in raw milk may be able to colonize the gut if consumed by humans, thus making infections difficult to treat. Evidence (Mahami *et al.*, 2011; Akoachere *et al.,* 2009) indicates that the global rise of antimicrobial resistance is mainly due to indiscriminate use of drug for treatment of both human and animal diseases.

Conclusion

The isolation of 10.5% *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 (96.4%) 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.

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