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Prevalence and antibiotic resistance patterns of *Salmonella* isolates from lactating cows and in-contact humans in dairy farms, Northwest Ethiopia

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ABSTRACT

Background: Salmonellosis is an infectious disease of humans and animals caused by species of *Salmonella*. *Salmonella* are widespread in the environment and commonly found in farm effluents and sewage. Epidemiological information on the prevalence of salmmonelosis is a prerequisite to develop appropriate control strategies. **Objective:** To assess the prevalence and antibiotic resistance patterns of *Salmonella* isolates. **Methods:** A cross-sectional study was conducted. A total of 152 cows and 14 humans were included in the study. Milk, fecal sample and human stool were collected. Samples were pre-enriched with buffered peptone water and transferred to selenite cysteine broth and rappaport-vassilidis broth. Isolation was made by plating on to xylose lysine deoxycholate agar and brilliant green agar. Biochemical, antibiotic susceptibility testing and serotyping were done. Data were entered and analyzed using SPSS version 20 software. Binary logistic regression was applied and p-values of less than 0.05 were considered statistically significant. **Results:** Of the total 21 *Salmonella* isolates identified, 19(90.5%) and 2(9.5%) isolates were from lactating cows and in-contact individuals, respectively. *Salmonella* bredeney was the most common (85.7%). Drinking water source had statistically significant (P=0.027) association. All isolates were resistant at least to one of the antibiotics tested with 100% to ampicillin. All isolates were sensitive to ciprofloxacin and amoxacilin/clavulanic acid. **Conclusion:** Large percentage of *Salmonella* isolates were resistant at least to one of the antibiotics tested. Salmonellosis treatment could be difficult in the future unless wise use of antibiotics is practiced.

KEY WORDS: *Salmonella;* Antibiotic resistance; Lactating cows; Gondar town; in contact humans

INTRODUCTION

Salmonellosis is an infectious disease of primarily of animals which can be transferred to humans. It is caused by one or more of the 2000 serotypes of the two species of *Salmonella* (*Salmonella enterica*, and *S. bongori*). *Salmonella* are facultative anaerobic gram-negative rods within the family of *Enterobacteriaceae*. The members of this genus are motile by means of peritrichous flagella except *Salmonella gallinarum* [1].

Although *Salmonella* are primarily intestinal bacteria, they are widespread in the environment and commonly found in farm effluents, human sewage and materials subjected to fecal contamination. *Salmonella* species are most important causes of acute gastroenteritis in several countries and salmonellosis remains an important public health problem worldwide, particularly in the developing countries [1, 2]. Approximately, 93.8 million human cases of gastroenteritis and 155 000 deaths occur due to *Salmonella* infection around the world each year [3]. According to center for disease control and prevention (CDC) analysis, *Salmonella* accounted for 25.6% of hospitalizations and 30.6% of deaths due to known food borne pathogens [4]. Even though non-typhoidal *Salmonella* (NTS) are among the most common causes of blood stream infections in under five children, there is shortage of epidemiological information in Ethiopian [5]. Recent cross sectional study conducted in Addis Ababa on dairy farms reported that 10.7% of lactating cows and 13.6% of the human subjects who attend the cows were shedding *Salmonella* [6].

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 [7]. The fecal matter from infected animals and humans are important sources of bacterial contamination of the environment and food sources [8].

Introduction of *Salmonella* onto a dairy farm can occur through a variety of routes, including purchased cattle, contaminated feed or water, wild animals such as rodents and birds, and human traffics. Human get infected when eating food or water contaminated with *Salmonella* through animal reservoirs [9].

Decade ago, there has been an alarming increase in the prevalence of antibiotic resistant *Salmonella* that may be a consequence of selective pressure associated with the use of antimicrobial agents in the feed of animals [10]. Studies have documented the role of foods of animal origin as a source of multi drug resistant *Salmonella* serovars [11, 12]. Antimicrobial usage in the feeds of animals are often at sub-therapeutic level or prophylactic doses which may promote on-farm selection of antibiotic resistant strains and markedly increase the risk of infection associated with consumption of contaminated meat and milk products [11, 13].

According to the 2006 United States of America (USA) Food and Drug Administration (FDA) report, nearly 23% of non-typhoid *Salmonella* isolates from humans were resistant to at least one antimicrobial agent and the most common multidrug resistance phenotype was reported to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline. On the veterinary side, 44% of the *Salmonella* isolated from animal slaughter and veterinary diagnostic sources were resistant to at least one antimicrobial agent and the phenotype resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracyclines was also the most common multidrug resistance profile [14].

Different factors are associated with increased risk of *Salmonella* shedding in dairies. Some identified factors associated with *Salmonella* prevalence in dairy farms are herd-size with large number of milking cows, free stall housing compared to other housing systems, use of flush water systems, hygienic conditions in farms both for cattle and humans, animal feeds as a source of *Salmonella* due to contamination like feeding brewers' products [15].

Different studies conducted in Ethiopia indicated considerable prevalence and antibiotic resistance patterns of *Salmonella* both in veterinary and public setups [6, 11, 16] and a single report from lactating cows and in contact humans [6]. But the representativeness of those studies to the country wide especially on lactating cows and humans attending them is limited. So the aim of this study was to determine the prevalence and antibiotic susceptibility patterns of *Salmonella* isolates from lactating cows and humans attending these cows in dairy farms in Gondar town.

METHODS AND MATERIALS

Study area, design and period

A cross-sectional study was employed from April 2013 to May 2013 at the governmental and private dairy farms in Gondar town. There is one governmental and 28 medium and small holder private dairy farms having lactating cows in the town. Gondar town is located 740 km Northwest of Addis Ababa in North Gondar administrative zone of Amhara regional state at an altitude range of 1500-2200 meter above sea level. The maximum and minimum temperatures of the area are 30.7°C and 12.3°C, respectively. The area receives a bimodal rainfall pattern with the annual precipitation rate being 1000 mm.

Study population

Selected lactating cows based on the sampling method and their volunteer in contact humans in selected governmental and private dairy farms in Gondar town were the study population. Lactating cows and in contact humans who were under antibiotic treatment were excluded from the study.

Operational definitions

Small size farm: A dairy farm with less than 25 heads.

Medium size farm: A dairy farm with 26 to 50 heads.

Off-farm food: Any food source for cows found outside of the farm like grazing fields.

Off-farm water: Any water source including flush water system found outside of the farm.

Clean farm: A farm in which cows are separately tied and feed, no manure contamination with food, periodically cleaned every day and dry.

Sample size and sampling technique

The sample size (n) was estimated by using the single population proportion formula Z^2 p [1-p) / d^2. Where P= prevalence of *Salmonella* from previous study in Addis Ababa 10.7% [6), d= margin of error (5%) and Z= standard score corresponds to 1.96 and 5% contingency were used which gives a sample size of 152. A total of 152 lactating cows and 14 volunteer in contact humans were in rolled in the study.

Sampling method

To select the lactating cows, the farms were stratified as government and private farms. A quota was then allocated for each farm with proportional allocation according to the number of lactating cows in each farm. As the number of lactating cows was manageable, we included all lactating cows in this study.

Data collection and laboratory processing

Information concerning in contact humans socio-demographic and associated factors was collected by the investigator through interview using a questionnaire. The questionnaire was originally developed in English and translated to Amharic. Questions related to educational background, farm size and cleanness, food and water source

of cows and were considered. To ensure consistency, the questionnaire was retranslated back to English by another person who was blind to the original one.

Samples from lactating cows and in contact humans were collected by using sterile containers. Approximately 25 gms of fecal specimens of cows were collected in a clean sterile airtight stool cup directly from the rectum. Approximately 20 ml milk samples were collected in a sterile universal bottle during usual milking time. About 15 gm of stool, which did not get in touch with the environment, were collected in sterile stool cup with an applicator stick from volunteer individuals working in the dairy farms. The samples were transported immediately after collection using an icebox to the University of Gondar faculty of Veterinary Medicine Microbiology Laboratory.

Laboratory processing and quality control

The isolation and identification of *Salmonella* was performed at the Microbiology laboratory of Faculty of Veterinary Medicine, University of Gondar, using techniques recommended by International Organizations for Standardization (ISO-6579, 2002), and those recommended by the World Health organization (WHO) Global food borne infections network (formerly WHO global *Salmonella* Surveillance) [28,29). The isolation and identification was involved the milk and fecal samples from lactating cows and human stool samples separately.

Non-selective pre-enrichment : Five gram of fecal, stool or 5 ml milk sample was transferred in to a plastic stomacher bag with 45 ml of buffered peptone water (BPW) (Himedia M1494I, India).The sample mixture was shaken with stomacher at 230 revolutions per minute for one to two minutes depending on sample type and incubated for 24 hrs at 37°C.

Selective enrichment: The pre-enrichment broth after incubation was mixed and a portion (0.1 ml) from each of the pre enriched culture was transferred to a tube containing 10 ml of selenite cysteine (SC) (Himedia MM025, India) broth and another 0.1 ml portion was transferred to a tube with 10 ml of Rappaport and Vassilidis (RV) (Himedia M1491, India) broth and incubated at 37°C and 42°C for 24 hrs, respectively.

Plating out and Identification: From each selective enrichment broths [Sc and RV), 10 μl of loop full sample was inoculated on-to xylose lysine deoxycholate (XLD) (Himedia M031, India) agar and brilliant green (BGA) (Oxoid CM0263, England) agar and incubated at 37°C for 24 hrs. Distinctive *Salmonella* colonies, showing red colonies with black center on XLD and red colonies on BGA were preserved with nutrient agar (Oxoid CM0003, England) slants using screw cupped test tubes in deep freezer by checking daily.

Biochemical confirmation: By using an inoculating loop, the following biochemical confirmation media were inoculated with the pure culture obtained in nutrient agar plates. Triple sugar iron agar (TSI) (Oxoid CM0277, England), Christensen's urea agar (Oxoid CM0053 England), lysine iron agar (LIA) (Oxoid CM0381, England), Voges Proskauer (VP) (Oxoid CM0043 England), citrate (Oxoid CM0155, England) and Indole tests (Oxoid CM0469, England). Those isolates tentatively identified as *Salmonella* were sub-cultured to brain heart infusion agar and sent to public health agency of CANADA (PHAC) for complete serotyping. The serotyping was performed using the WHO/OIE Reference ***methodology***. The O or somatic antigens of *Salmonella* isolates are detected by use of a slide agglutination method and the H or flagella antigens are detected by means of a microtitre plate technique and the antigenic formulae of Grimont and Weill was used to designate the serovars.

Antibiotic susceptibility testing : Finally, the antibiotic susceptibility tests of the *Salmonella* isolates were performed according to the National Committee for Clinical Laboratory Standards (NCCLS) method using Kibry-Bauer disk diffusion test on Muller-Hinton agar (Oxoid CM0337, England) [30). Pure colonies on nutrient agar were taken with a wire loop and transferred to a tube with saline and emulsified. Turbidity of bacterial suspensions were adjusted using McFarland standards as a comparator so that the number of *Salmonella* were within a given range and inoculated evenly on Muller Hinton agar plate with sterilized cotton top applicator stick. Then, the commonly used antibiotics were added, namely, ampicillin (10 μg), gentamycin (10 μg), ciprofloxacin (5 μg), ceftriaxone (30 μg), sulphamethoxazole-trimethoprim (25 μg), tetracycline (30μg), kanamycine (30 μg), nalidixic acid (30 μg), amoxycillin-clavulanic acid (30 μg) (oxoid, England) and cefotaxime (30 μg) (Benex, USA), and incubated for 24 hrs at 37°C. The diameter of zones of inhibition was documented to the nearest millimeter and classified as sensitive, intermediate and resistant according to standard tables published by Clinical and Laboratory Standards Institute [31].

All culture media were prepared based on the manufacturer's instruction and the prepared media were checked for sterility by incubating at 37OC for one day. All steps of the procedure were done strictly and aseptically as much as possible. Completeness of the questionnaires was checked whether important information was filled or not before data entry.

Data analysis

The data were entered and analyzed using the statistical package for social sciences (SPSS) version 20 software. Odds ratio was used to assess strength of association between different risk factors with prevalence of *Salmonella*. Binary logistic regression analysis was used to estimate the effects of each factor on *Salmonella* magnitude. Chi-squire test was

used to assess significant differences in antibiotic resistance between isolate of cows and humans as well as fecal and milk isolates of cows. The results with less than P-value of 0.05 were considered statistically significant.

Ethical considerations

The study was approved and ethical clearance was obtained from the ethical review committee of the School of Biomedical and Laboratory Sciences, University of Gondar. Informed consent was obtained from owners of the dairy farms and human study participants. Information that was obtained at each course of the study was kept confidential. In addition, the clinical specimens collected during the study period were used for the stated objectives only. Finally, positive individuals were given advice to go to clinic and owners of positive cows were communicated to take appropriate measures.

RESULTS

Prevalence of *Salmonella* in different farms

A total of 29 dairy farms, one governmental and 28 private were enrolled in this study and *Salmonella* were isolated from 13 (44.82%) farms. All isolated *Salmonella* were from the private dairy farms. In other words, among 28 private dairy farms examined, *Salmonella* were isolated from 13 (46.4%) of farms. Of the total farms included in this study, 17 were small and the rest 12 were medium size (Table 1).

From a total of 152 lactating cows examined in 29 dairy farms, 12.5 % (19/152) were positive for *Salmonella* either from milk or fecal samples or both (Table 2). The larger percentage of *Salmonella* were isolated from fecal samples (73.7 %) compared to 26.3 % from milk samples. From the cows confirmed *Salmonella* positive, one lactating cow was found positive from both milk and fecal samples. The rate of milk and fecal shedding of *Salmonella* by lactating cows were 3.3 % and 9.2 %, respectively. The difference in prevalence observed between milk and fecal samples of dairy lactating cows was not statistically significant ($\chi 2=0.524$, $P=0.469$). Stool samples were also collected and examined from 14 volunteer individuals among 29 interviewed in contact humans working in the dairy farms. The result indicated that 14.28 % (2/14) of volunteer persons who gave stool were positive for *Salmonella* (Table 2).

Table 1. Characteristics of farms in relation to size, ownership, age categories of cows and in contact humans in dairy farms in Gondar, Northwest Ethiopia, April 2013-May 2013.

Characteristic	Number	Percentage
Age range of cows		
5-10	79	52
>10	73	48
Size of farm		
Small	17	58.6
Medium	12	41.4
Ownership of farm		
Governmental	1	3.4
Private	28	96.6
Age range of humans		
<15	2	6.9
15-30	18	62.1
>30	9	31

Mean age of humans=24.4
Mean age of cows=10.1

Table 2. Prevalence of *Salmonella* from dairy farm lactating cows and in contact humans in Gondar Town, Northwest Ethiopia, April 2013-May 2013.

Sample type	Farm size		χ^2 (p-value)
	Small	Medium	
Milk of cows			
Positive	2 (2.3)	3 (4.5))	0.578 (0.447)
Negative	84 (97.7)	63 (95.5)	
Total	86 (100)	66 (100)	
Faeces of cows			
Positive	8 (9.3)	6 (9.1)	0.002 (0.964)
Negative	78 (90.7)	60 (90.9)	
Total	86 (100)	66 (100)	
Stool of humans			
Positive	2 (25)	0 (0)	1.750 (0.186)
Negative	6 (75)	6 (100)	
Total	8 (100)	6 (100)	

Among the total of 21 *Salmonella* isolates, 18 (85.7%) were *S. bredeney* and 3(14.3%) were S. Uganda. All *Salmonella* identified from milk sample and in contact humans were *S. bredeney* (Table 3).

Table 3. Distribution of *Salmonella* serotypes from milk, faecal of cows and stool of in contact humans in Gondar, Ethiopia.

Serotype	Antigen	Sample type			Number	Prevalence (%) (n=21)
		Milk	Faecal	Stool		
S. Bredeney	4:l, v:1,7	5	11	2	18	85.7
S. Uganda	10:l, z13:1,5	-	3	-	3	14.3
Total		5(23.8)	14(66.7)	2(9.5%)	21(100)	100

Risk factor for *Salmonella* infection

Information on management practices used on Gondar town dairy operations were collected and analyzed for association with shedding of *Salmonella* by dairy lactating cows. From 29 interviewed individuals working as a milker in the dairy farms about their hand washing habits, 82% of them have no the habit of hand washing before and after milking from which two individuals were diagnosed *Salmonella* positive. Data concerning about udder washing and towel use before milking was assessed using questionnaire and it was practiced in a governmental farm using one towel for one cow from which no *Salmonella* was isolated in all sample types. In the rest of all private farms, these activities were not practiced at all.

Regarding food and water sources of the cows, the information obtained from the questionnaire revealed that 64.5% of cows were served off-farm water source and the rest 35.5% were using clean (pipe) water in the farm. From all lactating cows included, only 25.7 % were feed with purchased feeds coupled with off-farm feeds and the remaining 74.3% were feed with off-farm food sources. Cleanness of farms was also observed and only 57 cows (37.5%) were live in clean farms and the rest 95 (62.5%) were living in none clean farms. Isolation rate of *Salmonella* between samples of cows in clean and none clean farms had not statistically significant (P=0.688) difference (Table 4).

The detection rate of *Salmonella* among lactating cows was highest at the age group greater than 10 in which 52.6% of the positives were observed among 73 cows. The rest, 47.4% was isolated in the age group 5-10 among 79 cows. The difference in prevalence in different age groups detected was not statistically significant ($\chi 2=0.184$, P=0.67).

According to the statistical analysis of different predictor variable for salmonellosis among dairy farm of lactating cows, there is statistically significant (p=0.027) difference in detection rate of *Salmonella* between lactating cows those drink clean (pipe) water in the farm and those drink any water source outside the farm (Table 4). The cows get water outside the farm are more than 5 times at risk of *Salmonella* than those obtained from the farm (OR=5.457). The rest predictor variables for *Salmonella* infection like farm size and food source for cows did not show statistically significant (p> 0.05) difference in *Salmonella* isolation among lactating cows.

Antibiotic susceptibility pattern

All of the 21 isolates of *Salmonella* from human and lactating cows were tested against ten commonly used antibiotics. The antibiotic susceptibility profiles of the isolates showed that all the isolates were 100% and 95.2% resistant to ampicillin and tetracycline, respectively. On the other hand, all isolates were 100% sensitive to amoxacycilin/ clavulanic acid and ciprofloxacin. In addition, kanamycin, gentamicin, ceftriaxone and cefotaxime were 95.2%, 90.5%, 90.5%, and 85.7% effective, respectively (Table 5).

All isolates were resistant at least to one antibiotic out of the ten antibiotics examined and the highest percentage of isolates (76.2%) showed resistance to two antibiotics tested (Table 6). The two *Salmonella* isolates from milk and fecal samples of a cow showed 100% similarity in antibiotic susceptibility test response. Resistance patterns observed between human and cow isolates as well as milk and fecal isolates of *Salmonella* subjected against a panel of ten antibiotics had no statistically significant (p>0.05) differences for each antibiotic.

Table 4. Risk factors for Salmonella infection among lactating dairy cows in Gondar Town, Northwest Ethiopia, April 2013-May 2013.

Risk factor	Salmonellosis status of cows		Total (%)	OR (95% CI)	P-value
	Positive (%)	Negative (%)			
Size of the farm					
Small	10 (6.6)	76(93.4)	86(56.6)	1.680(0.546, 5.223)	0.363
Medium	9 (5.9)	57(94.1)	66(43.4)		
Water source					
Clean water in farm	2 (3.7)	52(96.3)	54(35.5)	5.457(1.21, 24.602)	0.027
Off-farm water	17 (17.3)	81(82.7)	98(64.5)		
Farm cleanness					
Clean	3 (5.3)	54(94.7)	57(35.7)	1.377(0.289, 6.556)	0.688
Not clean	16 (16.8)	79(83.2)	95(64.3)		
Food source					
Purchased food	4 (10.3)	35(89.7)	39(25.7)	1.237(0.384, 3.991)	0.092
Off farm food	15 (13.3)	98(86.7)	113(74.3		

Table 5. Antibiotic susceptibility profiles of *Salmonella* isolates from dairy cows and in contact humans in Gondar Town, Northwest Ethiopia, April 2013-May 2013.

Antibiotics	Antibiotic susceptibility patterns		
	Sensitive	Intermediate	Resistant
Ampepicillin	0(0.0)*	0(0.0)	21(100)
Tetracycline	0(0.0)	1(4.8)	20(95.2)
Amoxacylin/clavulanic acid	21(100)	0(0.0)	0(0.0)
Ceftriaxone	18(85.7)	3(14.3)	0(0.0)
Cefotaxime	19(90.5)	2(9.5)	0(0.0)
Ciprofloxacin	21(100)	0(0.0)	0(0.0)
Gentamicin	19(90.5)	0(0.0)	2(9.5)
Kanamycin	20(95.2)	0(0.0)	1(4.8)
Nalidixic acid	16(76.2)	3(14.3)	2(9.5)
Sulfamethoxazole+ trimethoprim	18(85.7)	3(14.3)	0(0.0)

*Values in parenthesis are percentages

Table 6. Multiple antibiotic susceptibility patterns of *Salmonella* isolates from lactating cows and in contact humans in dairy farms of Gondar Town, Northwest Ethiopia, April 2013-May 2013.

No of antibiotic resistance	Antibiotics (number of isolates)	No of isolates (%)
R2	AMP,TE (16)	16 (76.2)
R3	AMP,TE,NA (2) TE,AMP,GE (1)	3 (14.3)
R4	AMP,TE,GE,KA (1)	1 (4.8)

Key: AMP (ampicillin), TE (tetracycline), NA (nalidixic acid), GE (gentamicin), KA (kanamycin),
R (resistant)

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. Detection of infected animals is essential to on-farm *Salmonella* control and its spread to the public [1, 2].

The current cross sectional study was designed to isolate *Salmonella* among lactating dairy cows and in contact humans in Gondar town. From 152 lactating dairy cows examined, 19 (12.5%) were found positive for *Salmonella* which is a little bit larger than a similar report in Addis Ababa (10.7 %) [6]. The difference in prevalence might be due to the use of, additional media, briliant green agar, which is recommended in the laboratory protocol for isolation of *Salmonella* species from food and animal faeces by WHO and ISO-6579, 2002 [28] in the present study. *Salmonella* prevalence from lactating cows (milk and fecal samples) obtained in this study was also higher than the prevalence recorded in Iran 4% [19] and in USA 7.3% [18]. Another research in Addis Ababa reported a prevalence of 14.7% from minced beef, mutton and pork samples among supermarkets [16] which is a bit larger than the current study and another study documented a slight less prevalence (11.5%) among chicken table eggs at Combolcha, Ethiopia [27], even though the sample sources are different from the current study.

Fecal prevalence of *Salmonella* among lactating dairy cattle in the current study was reported to be 9.2% which is interestingly similar with the fecal *Salmonella* isolation rate of 9.7% in United States [7]. However, a study from central Texas, USA [17] reported 70% (35/50) *Salmonella* shedding rate from fecal samples of dairy calf which is extremely higher than the present study. This extreme difference in *Salmonella* shedding rate might be in the report from Texas, 22 of 35 isolates were one serotype (S. Kinshasa) and this serotype might have specific host requirement. On the other hand the fecal carriage documented in Addis Ababa [6] is lower than the current study.

The two *Salmonella* serotypes isolated in the current study totally differs from a study conducted in Namibia [22] reported 29 serotypes other than the present report and a study reported 21 serotypes in Senegal [23] from which only four were *S. bredeney*.

This study also disagrees with a report of 1.56% prevalence in Egypt on fecal shedding of *Salmonella* among dairy cattle [24] and a bit higher than 10.9% reported in Namibia on bovine and ovine bone-and-meat meal and blood meal samples [22].

Furthermore, in the current study *Salmonella* isolation rate among individuals working in dairy farms were 14.28% (2/14) which was almost comparable with a report in Addis Ababa 13.63% among 22 dairy farm workers [6] but higher than a study reported zero percent in Egypt among 12 dairy farm attendants [24]. The difference in *Salmonella* prevalence among in contact humans might be due to difference in the hygienic condition of the farms and individual as well as awareness level about bacterial transmission in the farm.

Food handlers play vital role in the transmission of *Salmonella* species. A study conducted in Nigeria to determine the prevalence of *Salmonella* species reported 17% and 15% prevalence from apparently healthy food handlers and cattle, respectively [26] which were higher than the result of the present study in both sample sources.

Assessment of drinking water source for dairy lactating cows revealed that 64.5% were used any water source outside the farm and the rest 35.5% were used clean pipe water in the farm. This difference of water source for cows had statistically significant ($p=0.027$, OR=5.457 with a 95% CI 1.21, 24.602) association with the isolation rate of *Salmonella*. This result is in parallel with a study in America [15] reported that use of flush water system was identified as the most important predictive risk factor for *Salmonella* infection.

Antibiotic resistance in *Salmonella* is a rising problem during the last times. Improper use of antibiotics in both human

and veterinary medication has caused bacteria to develop resistance against therapeutic antibiotics [6, 19]. In this study, all the isolates showed 100% resistance to ampicillin and 95.2%% to ampicillin and tetracycline. This result is almost similar with a report from Addis Ababa, Ethiopia [6] and Lagos, Nigeria [26]. However, resistance rates to ampicillin and tetracycline is very high compared to results documented in America [18] reported 95.6% and 87.8% sensitivity levels, respectively and Iran [19] reported 42.58% sensitivity for both antibiotics. Highest resistance levels observed in the present study for the two antibiotics might be due to none judicious use of antibiotics in dairy farm level compared to reports from the above civilized countries.

Results of the current study indicated that ciprofloxacin and amoxacyline-clavulanic acid were 100% effective against *Salmonella* isolated from lactating cows and humans. This finding is in agreement with earlier reports in Turkey on *Salmonella* serotypes isolated among apparently healthy slaughtered sheep which showed 100% sensitivity [20] and in line with a report in Iran in which ciprofloxacin was 100% effective even though the sample sources was different in the later one with the present study [19].

Antibiotics tested in the current study like kanamycin, gentamicin, cefotaxime, ceftriaxone and sulphamethoxazole+trimethoprim indicated good activity against *Salmonella* isolates with 95.2%, 90.5%, 90.5%, 85.7% and 85.7% levels, respectively (Table 4). This is almost similar with a research report by Blau et al. [18] from United States on dairy operations which recorded 99.3% susceptibility levels to both kanamycin and gentamycin and slightly less sensitive to ceftriaxone and sulphamethoxazole+trimethoprim which showed 97.6% and 100% activities, respectively in the same research [18]. Furthermore, almost similar antibiotic susceptibility profile was observed with gentamicin, ceftriaxone and cotrimoxazole compared with similar study in Addis Ababa, Ethiopia, [6].

A study in Alexandria Egypt [24] reported that each of ampicillin and tetracycline were 85.7% effective against *Salmonella* species isolated in dairy cattle. This result strongly disagrees with the current study in which ampicillin and tetracycline were 100% and 95.2% resisted, respectively. On the other hand, the former study documented 100% resistant to sulphamethoxazole+trimethoprim which was 85.7% effective in this study. High activities of gentamicin and cotrimoxazole observed in the current study disagrees with a study in Texas, USA, [17] reported 85% and 80% resistance levels, respectively and this resistance difference might be these antibiotics are not easily accessed and over used in the current study farm setups.

In the current study, ciprofloxacin was 100% effective against both cow and human isolates. This finding is similar with a report in Sudan [25] in which ciprofloxacin was 100% effective to all human and cattle *Salmonella* isolates.

CONCLUSION

The current study presented considerable prevalence of *Salmonella* in lactating dairy cows and in contact individuals. 12.5% among lactating dairy cows and 14.28% from in contact individuals were recorded. All the isolated *Salmonella* were from private dairy farms in which proper hygienic condition were not practiced. Among all dairy farm workers interviewed, 82% had no the habit of hand washing before and after milking of cows from which two individuals were *Salmonella* positive. On the other hand, only 35.5% of cows were given clean water for drinking in farms and the remaining were using any water source outside the farms. It was found that using off-farm water as drinking source for cows increased the chance of *Salmonella* isolation rate compared to clean water source ($p=0.027$).

In this study, all the isolated *Salmonella* revealed resistance at least to one of the antibiotics tested. High percentage of multiple resistances to the commonly prescribed antibiotics was also observed. This may result treatment failure against salmonellosis. In general, results obtained in this study call for farther investigation on *Salmonella* prevalence in different farm settings together with serotyping and risk factor identification for emergence of multiple resistances to the commonly prescribed antibiotics.

COMPETING INTERESTS

The authors declare that they have no competing interests

AUTHORS CONTRIBUTIONS

DH and AG conceived the idea for this study. DH, AG and WM participated in the design and conduct of the study. DH, AG and LG were responsible for the accuracy of the data. DH, AG, WM drafted the manuscript. DH and AG guarantee the statistical analysis. All authors read and approved the final manuscript.

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