Prevalence and Antimicrobial Resistance Pattern of *Salmonella* Species from Foods of Bovine Origin in Dessie and Kombolcha Towns, Ethiopia

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Article

Prevalence and Antimicrobial Resistance Pattern of *Salmonella* Species from Foods of Bovine Origin in Dessie and Kombolcha Towns, Ethiopia

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Abstract: Bacteria are the major pathogens affecting food safety and foods of animal origin are main vehicles of human illness since food animals are the main reservoirs for many food-borne pathogens. Moreover, emergence and spread of multidrug-resistant food-borne bacterial pathogens become a significant public health concern globally. A cross-sectional study was conducted from October 2019 to July 2021 to estimate the prevalence, identify associated factors, and determine antibiotic resistance pattern of *Salmonella* species from foods of bovine origin in Dessie and Kombolcha towns. A total of 384 samples were collected. Simple and systematic random sampling techniques were employed for sampling milking cows and carcasses among cattle slaughtered at abattoirs, respectively. Samples from milk tanks, milk products, and beef were also selected randomly. *Salmonella* species were isolated and identified according to recommended standard bacteriological protocols. All the detected *Salmonella* species isolates were screened for *in vitro* antimicrobial susceptibility using agar disc diffusion method against 12 antimicrobial disks. The collected raw data were analyzed using descriptive and inferential analysis techniques. The overall prevalence rate of *Salmonella* species was 7.0%. The highest prevalence rate of *Salmonella* species (16.7%) was obtained from milk tank samples but not detected in milk products. Multidrug resistance to three and more than three drugs was observed among all isolated *Salmonella* species. All *Salmonella* species isolates (100.0%) were found to be resistant to Erythromycin, Tetracycline, and Vancomycin. The majority of the isolates (96.3%) were also resistant to Doxycycline and Polymyxin B. On the other hand, all isolates (100.0%) were sensitive to Gentamicin and Ciprofloxacin. The detection of multidrug-resistant *Salmonella* species showed that foods of bovine origin produced in the study sites were not safe for consumption. Hence, preventive measures are required to reduce bacterial contamination, concurrently to improve the wholesomeness and safety of foods of bovine origin.

Keywords: antibiotic resistance; Dessie; Kombolcha; prevalence; *Salmonella* species

1. Introduction

The consumption of foods of animal origin is increased from time to time due to globalization, rapid human population growth, urbanization, per capita income raise, and consumer desire for high protein diets [1–3]. With increasing consumption of products of animal origin, the risk of food-borne diseases of humans also increases [4] as food-producing animals are the major reservoirs for many food-borne pathogens [3].

Cow milk and its products may harbor a variety of microorganisms that can be important sources of food-borne pathogens [5]. Milk can be contaminated with microorganisms in many ways at different stages of production [4–8]. Similarly, meat and its products are important reservoirs for many of the food-borne pathogens which may cause food poisoning and human illness and bacterial contamination of these food items may occur from different sources at different stages of production chain [9–12].

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Bacterial pathogens are the foremost serious concern for public health from biological hazards [13]. Among food-borne bacterial pathogens, the genus *Salmonella* is one of the most important causative agents of gastroenteritis in humans and animals around the world, especially in developing countries [14–19]. In addition to human and animal morbidity and mortality costs, trade restrictions and disposal of contaminated food are important socio-economic problems of the bacteria [20].

Farm animals, including cattle, are the major reservoirs of non-typhoidal *Salmonella* serovars [21–23]. Foods of animal origin (milk, milk products, meat and its products) are the most common sources and vehicles of *Salmonella* infection in humans [1,24,18] and contamination of these products with *Salmonella* species can occur at multiple stages along the food chain [22].

On the other hand, antimicrobial resistant bacteria which can cause increased human morbidity and mortality are biological hazards having serious public health concern worldwide [25,26]. The widespread use of the antimicrobial agents in modern food-animal production system [27] contributed immensely to the emergence and spread of resistant bacteria and/or resistance genes that can be transmitted to humans through the food chain [25,26]. Antibiotic-resistant *Salmonella* species have been isolated from foods of bovine origin across the world and the occurrence of multidrug resistant *Salmonella* species in dairy and beef products has a significant impact on food safety [28,29]. The high prevalence and spread of multidrug-resistant *Salmonella* serovars are universal public health concerns, particularly in developing countries [30–32].

The prompt and precise identification of bacterial pathogens in food is critical for tracing bacterial pathogens within the food chain as well as ensuring food quality and safety [33]. However, the actual magnitude and incidence as well as antimicrobial drug resistance condition of major food-borne diseases in most developing countries is not well known because of absence of national surveillance and monitoring programs, and poor or non-existent reporting system [34]. In most parts of Ethiopia, cow milk and beef are consumed as raw or under cooked and raw milk is used as a starting material for preparing dairy products such as yoghurt, butter, and buttermilk. Thus, there exists the possibility of consuming milk and beef which have been contaminated with disease causing multidrug resistant bacteria including *Salmonella*. Hence, the current study was aimed by taking into account the great public health significance of *Salmonella* species, and the high beef and dairy cattle population in the area as well as community's consumption habit of animal products. Therefore, the objectives of the present study were to estimate the prevalence and identify associated factors of *Salmonella* species from foods of bovine origin in Dessie and Kombolcha towns and to determine the antibiotic resistance patterns of *Salmonella* species.

2. Materials and Methods

2.1. Ethics Approval and Consent to Participate

This study was reviewed and approved by the Research Ethics Committee of the School of Veterinary Medicine, Wollo University. Owners, managers, and workers of the different sites were informed of the procedures and significance of the study. Each data and analysis result was kept confidential and communicated to concerned bodies. Any participants who were not volunteers were not forced to be included.

2.2. Study Area

The study was conducted in Dessie and Kombolcha towns of South Wollo Zone, Eastern Amhara Region, Ethiopia as shown in Figure 1. Dessie is the capital city of South Wollo zone which is 401km far from Addis Ababa, the capital city of Ethiopia. Its geographical location is at 11°8'N-11°46'North latitude and 39°38'E-41013'East longitude with an elevation between 2,470 and 2,550 meters above sea level. The town is bounded by Kutaber Woreda in the north, Dessie Zuriya Woreda in the east and by Kombolcha town in the south. The topography of Dessie is a highland type surrounded by 'Tossa' mountain. Annual maximum and minimum temperatures of Dessie town are 23.7°C and 9°C, respectively. It has a mean annual rainfall of 1100-1200 mm. Dessie is one of the reform towns in the region and has a city administration consisting of municipality 26 kebeles, 18 urban and 8 rural [35].

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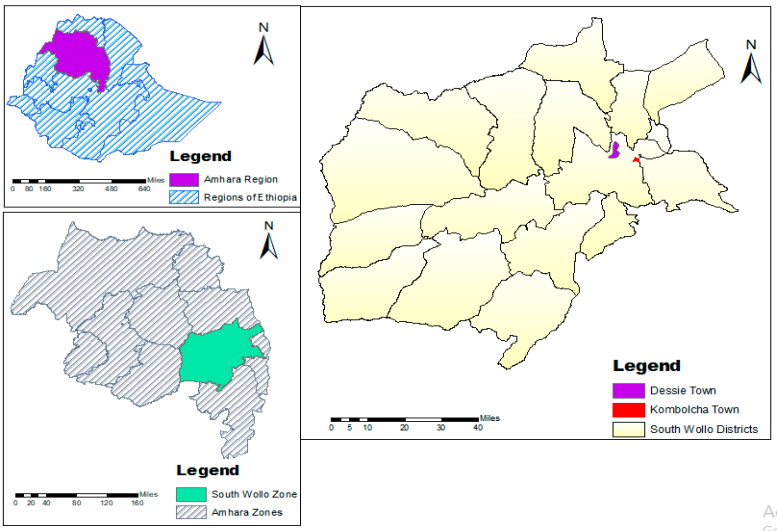

Figure 1. Map of the study areas.

Kombolcha is an industrial town found in the north-central part of Ethiopia in South Wollo Zone of the Amhara Regional State, Ethiopia. The town is situated at a distance of 23 km from the zonal town, Dessie, 505 km from the Regional capital city, Bahirdar, 376 km from north of Addis Ababa and 533 km from port Djibouti. Geographically, the town is located at about 11°6′ N latitude and 39°45′E longitudes. The town is located in a range of altitudes between 1, 500 and 1, 840 meter above sea level. The delimitation of the town is bounded by Dessie Zuria Woreda in the North East and North West, Kalu Woreda in the South and Albuko Woreda in the South West [36]. Mean annual rainfall is 1046 mm while annual maximum and minimum temperatures are 28.1°C and 12.9°C, respectively. Kombolcha is one of the reform towns in the region and has a town administration municipality, 5 urban and 6 peri-urban kebeles [37].

2.3. Study Population

Study samples of foods of bovine origin were selected from dairy farms, milk product shops, municipal and ELFORA abattoirs, butcher shops, and restaurants in the study areas. Two municipal abattoirs, one in each town, are found in the study areas. Averagely, 5 oxen were slaughtered per day in Dessie municipal abattoir and 30 to 35 oxen were slaughtered on Friday of each week. Though Dessie municipal abattoir had 90 registered customers, only 27-30 of them were active customers. In average, two oxen were slaughtered per day at Kombolcha municipal abattoir due to expansion of illegal field slaughtering practice and administrative problems. In both municipal abattoirs, there was no clear division of slaughtering process: stunning, bleeding, skinning, evisceration and carcass splitting area. There was no overhead rail in Kombolcha municipal abattoir and slaughtering process was conducted on floor of one room. In Dessie municipal abattoir, carcass was hanged for splitting on overhead rail following bleeding, skinning and evisceration on the floor. On the other hand, Komblolcha ELFORA abattoir was equipped with most of facilities and slaughtering process was conducted in separated areas of the abattoir. According to the meat inspector (veterinarian) report and confirmed during supervision, 80-140 (120 in average) cattle were slaughtered per day at ELFORA abattoir.

During the time of sample collection, 164 registered dairy farms were found in Kombolcha town. According to the well organized document of Kombolcha Town Animal Production and Health Office (2019) [38], the total milking, dry and pregnant cows were 586, 266, and 386, respectively. The daily average milk yield in the town was 6,261 liters. However, the documentation was poor in Dessie Town Animal Production and Health Office (2019) [39] and the officers gave a document of only seven large scale and well organized dairy farms in the town. Assessment immediately before sample

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collection revealed that around 28 dairy farms were found in the town excluding farmers having 1 to 2 milking cows. In these farms, the milking cows were around 196.

2.4. Study Design

A cross sectional study was conducted from October, 2019 to July, 2021 to estimate the prevalence and antibiotic resistance pattern of *Salmonella* species from foods of bovine origin in the selected study area.

2.5. Sample Size and Sample Collection

The sample size (n) was estimated using the statistical formula recommended by Thrusfield (2005) [40].

$$n = \frac{Z^2 Pexp(1-Pexp)}{d^2} \qquad n = \frac{1.96^2 Pexp(1-Pexp)}{d^2}$$

Where, n = sample size, z = statistic for a level of confidence
d = required absolute precision, P_{exp} = expected prevalence

For sample size calculation, 95% confidence interval, and 50% expected prevalence (Pexp) of *Salmonella* species with absolute precision (d) of 0.05 were used. Based on the above recommended formula, the minimum desired sample size was calculated to be 384. The sample size of each sample source was fairly distributed after knowing their total numbers in the study areas. A total of 384 foods of bovine origin samples, comprising of udder milk (146), bucket milk (6), cheese (9), yoghurt (36), carcass swab (162), and beef swab (25) from dairy farms, milk product shops, butcher shops and restaurants, and abattoirs were collected in two selected study areas. Among 384 samples, 181 were collected from Dessie town and the remaining 203 were collected from Kombolcha town.

2.6. Sampling Technique and Sample Collection

Simple random sampling technique was employed for sampling cows to collect udder milk samples and systematic random sampling method (every third animal was selected) was carried out to select carcass swab samples among cattle slaughtered at abattoirs in study sites. Similarly, random sampling technique was employed to select samples from milk tank and milk product shops, and beef swab samples from butcher shops and restaurants.

Milk and its product samples were collected aseptically using sterile labeled screw cupped glass bottles. From each selected milking cow, about 25 ml of milk sample was collected from all quarters of the udder at the middle of milking procedure following the milkers prepared the cows for milking through usual practice. Around 25 ml/g yoghurt and cheese samples were collected aseptically from milk product shops using sterile labeled screw cupped glass bottles. The carcass swab samples were collected using sterile cotton swabs from the surface and deep part of carcasses at five different sampling locations (neck, thorax, abdomen, breast and crutch) of selected slaughtered cattle. The swab samples from different locations of the same carcass were pooled together and placed into labeled test tubes containing 5 ml of sterile 0.85% NaCl solution. The beef swab samples were collected from different sites of the beef at butcher shops and restaurants, and the swab samples were transferred into labeled test tubes containing 5 ml of sterile 0.85% NaCl solution. The required information for each of the different sample types were recorded on prepared recording formats at the time of sample collection. The collected samples were transported in ice box containing ice packs to School of Veterinary Medicine Laboratory, Wollo University on the day of collection, stored aseptically and analyzed within 24 hours.

2.7. Isolation and Identification of Salmonella species

Salmonella species were isolated and identified according to standard bacteriological techniques for *Salmonella* detection recommended by FDA [41] and Quinn *et al*. (2002) [42]. All media required for *Salmonella* detection were prepared and used according to manufacturers' recommendations. For each collected sample, conventional culture methods based on non-selective pre-enrichment

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followed by selective enrichment, culturing on selective, differential and general purpose agar media, and standard biochemical tests were done for isolation and identification of *Salmonella* species. After mixed thoroughly, 1 ml of the original sample was inoculated in to 9 ml of sterile peptone water (Micromaster, India) and incubated aerobically at 37°C for 24 hours for pre-enrichment. For selective enrichment, 0.1 ml of mixed pre-enriched sample was transferred into test tube containing 10 ml of Rappaport-Vassiliadis broth (HiMedia Laboratories Pvt.Ltd., India) and incubated at 37°C for 24 hours.

A loopful of well mixed selective enrichment broth culture was streaked onto MacConkey Agar medium (HiMedia Laboratories Pvt.Ltd., India) through quadrant streak technique and plates were incubated at 37°C for 24 hours. The non-lactose fermenting colorless colony from the MacConkey culture plates was streaked onto the surface of nutrient agar plates (HiMedia Laboratories Pvt.Ltd., India), and incubated aerobically at 37°C for 24 hours. Single colony was picked up from nutrient agar and Gram's staining was performed as per procedures described by Merchant and Packer (1969) [43] to determine the Gram's reaction, shape and arrangement of bacteria. Catalase test was done by picking colony of the isolates using a sterile wooden stick form the nutrient agar plate and mixing with a drop of 3% H_2O_2. The colonies were further subcultured onto Salmonella-Shigella agar media (HiMedia Laboratories Pvt. Ltd., India) and all plates were then incubated aerobically at 37°C for 24 hours. After 24 hours, the plates were examined for the growth of characteristic *Salmonella* colonies (colorless colonies with black center) (Figure 2). The presumptive *Salmonella* colonies were further streaked on Xylose Lysine Desoxycholate (XLD) agar plates (HiMedia Laboratories Pvt.Ltd., India) and incubated at 37°C for 24 hours for the appearance of characteristic red colonies with a black center surrounded by a pink-red zone (Figure 3).

Figure 2. Growth on SS agar plates.

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Figure 3. Growth on XLD agar plates.

Colonies suspected to be Salmonella on the basis of Gram's reaction (pink colored with rod shape), catalase test (forming bubbles), cultural and morphological characteristics on selective media were subcultured nutrient agar (HiMedia Laboratories Pvt.Ltd., India) and subjected to selected biochemical tests for identification. Suspected Salmonella colonies were picked from the nutrient agar using an inoculating loop and inoculated into Tryptone broth (HiMedia Laboratories Pvt.Ltd., India) and Methyl Red-Voges Proskauer (MR-VP) broth (Guangdong Huankai Microbial Sci. &Tech.Co., Ltd, China). Similarly, colonies were inoculated into Simmon's citrate agar slants (HiMedia Laboratories Pvt.Ltd., India), Christensen's Urea agar slants (Microxpress, India), and Triple Sugar Iron (TSI) agar slants (Sisco Research Laboratories Pvt. Ltd, India) through stab and streak technique and incubated at 37°C for 24 hours for confirmation of identification [44,45].

All the phenotypically and molecularly characterized isolates of *L. monocytogenes* were tested for antibiotic susceptibility patterns. The method applied for the in vitro antimicrobial susceptibility testing of *L. monocytogenes* isolates was the agar plate antibiotic disk diffusion method using Kirby-Bauer technique [49]. The following thirteen antimicrobial disks (belong to eight classes of antimicrobials) (Himedia Laboratory Pvt Limited, Mumbai, India) with their concentrations given in parentheses were used in the antibiogram testing: Penicillin class antimicrobials (amoxicillin (25µg), ampicillin (10µg), cloxacillin (5µg), methicillin (30µg), and penicillin G (10µg)); Fluoroquinolones class antimicrobial (ciprofloxacin (5µg)); Lincomycin class antimicrobial (clindamycin (10µg)); Macrolide class antimicrobial (erythromycin (15µg)); Aminoglycoside class antimicrobials (gentamycin (10µg) and streptomycin (10µg)); Quinolone class antimicrobial (nalidixic acid (30µg)); Tetracyclineclass antimicrobial (tetracycline (30µg)); and Glycopeptides class antimicrobial (vancomycin (30µg)). The selection of these antimicrobials was based on the availability and frequent use of these antimicrobials in the study area both in veterinary and human medicine. Standard strains of L. monocytogenes ATCC 7644 and S. aureus ATCC 25923 were used as positive and negative controls, respectively. The results were interpreted as Susceptible (S), Intermediate (I), and Resistant (R) categories based on the critical points recommended by the Clinical and Laboratory Standards Institute (Additional file 1: Figure S7) [50].

After overnight incubation, 0.5 ml of Kovac's reagent (HiMedia Laboratories Pvt.Ltd., India) was poured in to Tryptone broth culture for indole test, 0.3 ml of 1% Methyl red solution (Dallul Pharmaceuticals Plc., Ethiopia) was dropped in to MR-VP broth culture for methyl red test, 0.6 ml of 5% alpha napthanol (Loba Chemie Pvt.Ltd, Mumbai, India) and 0.2 ml of 40% potasium hydroxide solution (Unichem Laboratories Ltd., India) was added in to MR-VP broth culture for Voges-Proskauer test and the result of each biochemical test was interpreted. Isolates producing acid (yellow color) butt and alkaline slant with hydrogen sulfide production on TSI, negative for indole test (no pink to red ring), negative for urea hydrolysis (remaining yellow color), methyl red positive (red

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color), Voges-Proskauer negative (no pink-red color at the surface), and positive for citrate utilization (blue slant) were confirmed to be *Salmonella* species [46].

2.8. Antimicrobial Susceptibility Testing of Salmonella species

All isolates of Salmonella identified in this study were screened for in vitro antimicrobial susceptibility using the agar disc diffusion method recommended by Bauer et al. (1966) [47]. Isolates were tested against the following twelve different antibiotic discs (Mast Group Ltd., Merseyside, U.K) with their concentrations given in parentheses: Erythromycin (15µg), Nalidixic acid (30µg), Kanamycin (30µg), Gentamicin (10µg), Amoxicillin (10µg), Doxycycline (30µg), Tetracycline (TE) (10µg), Penicillin G (P) (10 IU), Sulfamethoxazole-trimetoprim (25µg), Polymyxin B (300 IU), Vancomycin (VA) (5µg), and Ciprofloxacin (5µg).

Biochemically confirmed Salmonella species isolates were inoculated onto nutrient agar and incubated at 37°C for 24 hours. Colonies from an overnight culture grown on nutrient agar plates were transferred and diluted into test tubes containing 5 ml of sterile 0.85% saline solution and mixed vigorously to form a homogeneous suspension until the turbidity of the bacterial suspension achieved the 0.5 McFarland turbidity standards. Sterile cotton swab was immersed into the adjusted suspension and the excess inoculum was removed by lightly pressing the swab against upper inside wall of the test tube.

The swab containing the inoculum was then spread evenly over the entire surface of the Mueller-Hinton agar plate (HiMedia Laboratories Pvt.Ltd., India) to obtain uniform inoculums over the entire surface of Mueller-Hinton agar plate. After the inoculated plates dried for 3 to 5 minutes, antibiotic impregnated discs were placed on the agar surface using sterile thumb forceps and gently pressed with the point of a sterile forceps to ensure firm contact with the media surface. Four antibiotic discs were placed in each petridish at a minimum distance of 24 mm to prevent overlapping of the inhibition zones. Within 15 minutes of the application of antibiotic discs, the plates were inverted and incubated at 37°C for 24 hours. Following the overnight incubation, the diameters of the zones of growth inhibition around each of the antibiotic disk were measured using digital caliper and the results were recorded. The recorded results of inhibition zones around individual antibiotic disks were interpreted and the isolates were classified as Sensitive (S), Intermediate (I), and Resistant (R) according to the interpretation tables of the Clinical and Laboratory Standard Institute [48–51], Arabzadeh *et al*. (2018) [52], Reza *et al*. (2020) [53], Tadesse *et al*. (2018) [54], and TMCC (2021) [55].

2.9. Standard Strains for Quality Control.

The standard strain of *Salmonella enterica* obtained from Amhara Public Health Institute (APHI) Dessie branch was used as control strain to increase the confidences in the reliability of test results.

2.10. Data Management and Processing.

All collected raw data were compiled, organized, entered, and coded in Microsoft Excel 2007 spread sheet and transferred to STATA Version 12 software for statistical analysis. The collected raw data were analyzed using descriptive and inferential analysis techniques. Descriptive statistics such as frequency and/or percentage were calculated. In addition to proportion, chi-square test (χ^2) and P value were computed to see the association of risk factors with that of occurrence of *Salmonella* species isolates.

3. Results

3.1. Overall Prevalence

Out of the total of 384 examined samples of foods of bovine origin, 27 (7.0%) were found to be contaminated with *Salmonella* species (Table 1). The site based prevalence rates of *Salmonella* species in Dessie and Kombolcha towns were 6.6% and 7.4%, respectively. No statistically significant differences were observed in the prevalence rates of the bacteria between the two study sites (P>0.05).

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Among the examined sample types, highest (16.7%) and lowest (0.0%) prevalence rates of *Salmonella* species were recorded from milk tank and milk products, respectively. The difference in the prevalence of *Salmonella* species among different sample types was not statistically significant (P>0.05) (Table 1).

Table 1. Prevalence of *Salmonella* species among the sample types and study sites.

Variables	No. of examined	No. of positive (%)	χ^2-value	P value
Sample types				
Udder milk	146	8 (5.5)		
Tank milk	6	1 (16.7)		
Yoghurt	36	0 (0.0)	6.836	0.233
Cheese	9	0 (0.0)		
Beef swab	25	2 (8.0)		
Carcass swab	162	16 (9.9)		
Study sites				
Dessie	181	12 (6.6)	0.0844	0.771
Kombolcha	203	15 (7.4)		
Overall	**384**	**27 (7.0)**		

3.2. Prevalence of Salmonella Species among Variables of Different Sample Types

The highest proportion of *Salmonella* species in milk samples (66.7%) was isolated from dairy farms with poor milking practice and the difference was statistically significant (P<0.05) (Table 2).

Table 2. Prevalence of *Salmonella* Species among different variables of milk samples.

Variables	No. of examined	No. of positive (%)	χ^2-value	P-value
Study sites				
Dessie	52	6 (11.5)	4.477	0.034
Kombolcha	100	3 (3.0)		
Sample types				
Pooled Udder milk	146	8 (5.5)	1.295	0.255
Bucket milk	6	1 (16.7)		
Farm systems				
Intensive	131	9 (6.9)	1.534	0.216
Semi Intensive	21	0 (0.0)		
Treatment history				
No	50	4 (8.0)	0.578	0.447
Yes	102	5 (4.9)		
Milking practices				
Excellent	3	0 (0.0)		
Very good	42	0 (0.0	22.287	0.000
Good	104	7 (6.7))		
Poor	3	2 (66.7)		
Farm hygiene				
Excellent	4	0 (0.0		
Very good	52	1 (1.9)	2.759	0.430
Good	85	7 (8.2)		
Poor	11	1 (9.1)		
Total	**152**	**9 (5.9)**		

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According to the result presented in Table 3, there was no statistically significant difference in the prevalence of Salmonella species among all hypothesized variables of carcass swab samples (P>0.05).

Table 3. Prevalence of *Salmonella* Species among variables of carcass swab samples.

Variables	No. of examined	No. of positive (%)	χ^2-value	P-value
Study sites				
Dessie	96	6 (6.2)	3.482	0.062
Kombolcha	66	10 (15.2)		
Sources				
Municipal Abattoir	105	9 (8.6)	0.571	0.450
ELFORA	57	7 (12.3)		
Hygiene of slaughtering process				
Very good	78	7 (9.0)	0.447	0.800
Good	49	6 (12.2)		
Poor	35	3 (8.6)		
Hygiene of butchers				
Very good	78	7 (9.0)	2.765	0.251
Good	55	8 (14.5)		
Poor	29	1 (3.4)		
Hygiene of slaughtering materials				
Excellent	57	7 (12.3)	1.373	0.503
Good	83	6 (7.2)		
Poor	22	3 (13.6)		
Total	**162**	**16 (9.9)**		

The difference in the prevalence of Salmonella species among all hypothesized variables of beef swab samples was not statistically significant (P>0.05) as presented in Table 4.

Table 4. Prevalence of *Salmonella* Species among the variables of beef swab samples.

Variables	No. of examined	No. of positive (%)	χ^2-value	P-value
Study sites				
Dessie	13	0 (0.0)	2.355	0.125
Kombolcha	12	2 (16.7)		
Where get slaughtered				
Abattoir	21	1 (4.8)	1.870	0.171
Field	4	1 (25.0)		
Hygiene of butchers				
Very good	18	1 (5.6)	0.845	0.655
Good	6	1 (16.7)		
Poor	1	0 (0.0)		
Hygiene of cutting utensils				
Very good	5	1 (20.0)	1.223	0.269
Good	20	1 (5.0)		
Hygiene of butcher shops				
Excellent	2	0 (0.0)		
Very good	13	1 (7.7)	0.570	0.903
Good	8	1 (12.5)		
Poor	2	0 (0.0)		

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Total	**25**	**2 (8.0)**

3.3. In Vitro Antimicrobial Sensitivity Pattern

Out of the total of 27 *Salmonella* species isolates screened for antimicrobial sensitivity test against twelve antibiotics, all isolates (100.0%) were found to be resistant to Erythromycin, Tetracycline, and Vancomycin. Higher percentages of the isolates (96.3%) were also resistant to Doxycycline and Polymyxin B. On the other hand, all isolates (100.0%) were sensitive to Gentamicin and Ciprofloxacin as shown Table 5.

Table 5. *In vitro* antimicrobial sensitivity pattern of *Salmonella* species isolated from different sample types of foods of bovine origin.

Antimicrobial agents	Interpretation categories		
	Sensitive	**Intermediate**	**Resistant**
Erythromycin	0 (0.0)	0 (0.0)	27 (100)
Nalidixic acid	23 (85.2)	4 (14.8)	0 (0.0)
Kanamycin	19 (70.4)	8 (29.6)	0 (0.0)
Gentamicin	27(100)	0 (0.0)	0 (0.0)
Amoxicillin	21 (77.8)	0 (0.0)	6 (22.2)
Doxycycline	1 (3.7)	0 (0.0)	26 (96.3)
Tetracycline	0 (0.0)	0 (0.0)	27(100)
Penicillin G	6 (22.2)	21 (77.8)	0 (0.0)
Sulfamethoxazole-trimetoprim	22 (81.5)	0 (0.0)	5 (18.5)
Polymyxin B	1 (3.7)	0 (0.0)	26 (96.3)
Vancomycin	0 (0.0)	0 (0.0)	27(100)
Ciprofloxacin	27 (100)	0 (0.0)	0 (0.0)

All isolates of *Salmonella* species showed multidrug resistance to more than three drugs. Thus, 1(3.7%), 21 (77.8%), and 5 (18.5%) of the isolates were resistant to four, five, and seven drugs, respectively as shown in Figure 4.

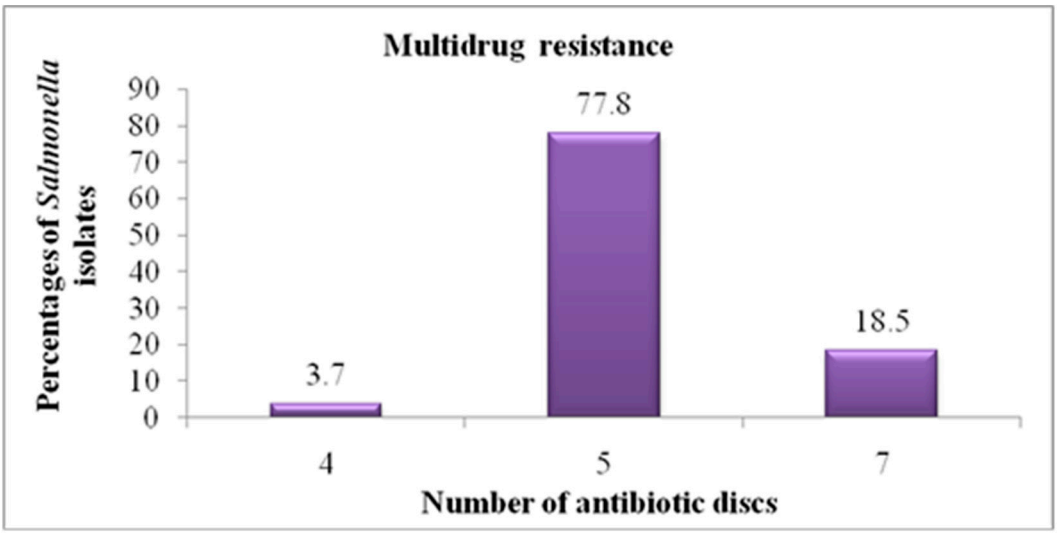

Figure 4. Multidrug resistance pattern of *Salmonella* species isolates.

4. Discussion

In the present study, the overall prevalence of *Salmonella* species was 7.0%. This prevalence was consistent with studies that have been reported by Abunna *et al*. (2018b) [56] in Meki Town (7.01%),

Atsbha *et al*. (2018) [57] in Mekelle city (7.29%), Singh *et al*. (2018) [58] in Jabalpur city (India) (7.61%), Bekele and Lulu (2017) [59] in Haramaya University abattoir (7.8%), Ejo *et al*. (2016) [28] in Gondar town (5.5%), Abunna *et al*. (2017) [60] in and around Modjo town (5.3%), Gebremedhin *et al*. (2021) [16] in Ambo and Holeta towns (5.7%), Karshima *et al*. (2013) [61] in Kanam (Nigeria) (8.7%), Alemu and Zewde (2012) [62] in Bahir Dar (4.8%), Rahman *et al*. (2018) [63] in Bangladesh (6.78%), Kalambhe *et al*. (2016) [64] in Nagpur region (Central India) (6.0%), Musa *et al*. (2017) [22] in Maiduguri (North-Eastern Nigeria) (10.0%), and Mulaw (2017) [65] in Bahirdar town (9.35%). Moreover, this finding was comparable with research findings reported from Addis Ababa by Banti (2018) [66] (6.0%), Alemayehu *et al*. (2003) [67] (7.1%), Zerabruk *et al*. (2019) [68] (6.25%), Addis *et al*. (2011) [15] (5.9%), and Kebede *et al*. (2016) (5.7%) [69].

The prevalence of *Salmonella* species from foods of bovine origin in the present study was higher than the reports of Mhone *et al.* (2012) [70] (0.0%) in Zimbabwe, Abunna *et al*. (2018a) (0.55%) [14] in Adama town, Hiko *et al*. (2015) (0.8%) [71] in Addis Ababa, Shilangale *et al*. (2015) (0.85%) [72] in Namibia, Chyea *et al*. (2004) (1.4%) [73] in Malaysia, Liyuwork *et al.* (2013) (1.6%) [74] in Addis Ababa, Sibhat *et al*. (2011) [32] (2.0%) in Debre Zeit town, Kore *et al*. (2017) [21] (2.0%) in Hawassa town, Ketema *et al*. (2018) (2.5%) [75] in Addis Ababa, Van-Kessel *et al*. (2004) (2.6%) [76] in the United States, Mengistu *et al*. (2017) (2.75%) [77] in Eastern Ethiopia, Beyene *et al*. (2016) (2.8%) [78] in Asella town, and Reta *et al.* (2016) (3.3%) [79] in Jigjiga city.

However, the prevalence of *Salmonella* species in the present study was lower than the reports from Bahir Dar city (70.0%) [80], Jigjiga city (20.8%) [19], Kersa District (Jimma Zone) (20.0%) [81], Gondar town (12.5%) [17], Debre Zeit (23.6%) [18], Jimma town (11.3%) [82], Addis Ababa (14.4%) [83], Mizan town (13.4%) [30], Madurai (South India) (13.3%) [84], and Addis Ababa (12.9%) [85]. The variations in the prevalence of *Salmonella* species between the present and previous studies reported in different areas of the Ethiopia and other countries abroad could be due to differences in study methods employed by the investigators (sample type, sampling techniques, sample size, sample sources, and methods of detection in laboratories), management and hygienic practices in dairy and beef farms, herd size, hygienic conditions in slaughter houses and milking premises, cleanliness of milking and slaughtering utensils, hygienic practices during milking and slaughtering, levels of cross-contamination, personal hygiene, water quality and its availability, and the methods and hygienic practices of handling, transportation, and storage of foods of bovine origin.

The present study showed that the prevalence of *Salmonella* species from tank milk, carcass swab, beef swab, udder milk, yoghurt, and cheese samples was 16.7%, 9.9%, 8.0%, 5.5%, 0.0%, and 0.0%, respectively. The difference in prevalence of *Salmonella* species among different sample types was not statistically significant ($P>0.05$). Relatively higher contamination of tank milk with *Salmonella* species could be due to either initial contamination of milk from milking process, equipment used for milking, personnel and/or further contamination of milk during collection in poorly cleaned tank.

The proportion of *Salmonella* species from milk samples was higher in Dessie town (11.5%) than Kombolcha town (3.0%) and the difference was statistically significant ($P<0.05$). This might be due to the difference in hygienic practices at dairy environment between the two study sites. The highest proportion of *Salmonella* species in milk samples (66.7%) was isolated from dairy farms with poor milking practice and the difference was statistically significant ($P<0.05$). This high proportion was not surprising since *Salmonella* species contamination of raw milk and its products is mostly caused by infected persons and environmental contamination, while natural udder infections are uncommon and seldom contribute to human food poisoning [70]. *Salmonella* species were not detected in milk products. According to Szczawiński *et al*. (2014) [86], *Salmonella* cells have unfavorable conditions for growth in yogurt as storage temperature and pH of yogurt significantly influenced survival rate of these bacteria.

The problem of antibiotic resistance has become a significant public health concern globally [87] and the rapid development of multidrug resistance hampered the effectiveness of treatments both in veterinary and public health sectors [88]. Numerous strains of *Salmonella* have been identified as resistant to multiple antibiotics which are currently considered as emerging food-borne pathogens [89]. In the current study, all isolates of *Salmonella* species showed multidrug resistance to more than

three drugs. Summarily, 3.7%, 77.8%, and 18.5% of the isolates were resistant to four, five, and seven drugs, respectively.

In the present study, all the isolates of *Salmonella* species (100.0%) were found to be resistant to Erythromycin, Tetracycline, and Vancomycin. A higher percentage of the isolates (96.3%) were also resistant to Doxycycline and Polymyxin B. The total resistance to Erythromycin was similar with earlier reports of Musa *et al*. (2017) [22] and Alemu *et al*. (2020) [30] who reported 100.0% Erythromycin-resistant *Salmonella* species isolates in Nigeria and Mizan town (Ethiopia), respectively. The resistance of all isolates to Tetracycline (100.0%) was consistent with the previous reports of Hailu *et al*. (2015) [17] in Gondar town and Abunna *et al*. (2017) [60] in Modjo town who reported 95.2% and 96.4% resistance to Tetracycline, respectively. However, Ekli *et al.* (2019) [90] reported the isolates which showed 100.0% susceptibility to Tetracycline in Wa Municipality of Ghana.

The extensive, indiscriminate and injudicious use of antibiotics both in human and veterinary medicine leads to genetic modification in most bacterial strains for evolving resistance and an increase in the prevalence of resistance among pathogens [91,92]. Thus, the high resistance pattern of the *Salmonella* species isolates to the readily available and relatively inexpensive antibiotics might be due to extensive use of these antibiotics for long period of time in the community as well as dairy and beef cattle production sectors.

On the other hand, the isolates of *Salmonella* species were totally sensitive to Gentamicin and Ciprofloxacin. The sensitivity of all isolates to Gentamycin was similar with previous studies that reported 100.0% susceptibility to Gentamycin from Mizan town [30], Addis Ababa [74], Adama town [14], Asella town [78], and Modjo town [60]. The susceptibility of all isolates to Ciprofloxacin was similar with earlier reports of Banti *et al*. (2018) [66] and Liyuwork *et al*. (2013) [74] in Addis Ababa town, Beyene *et al*. (2016) [78] in Asella town, Abunna *et al*. (2017) [60] in Modjo town, Takele *et al*. (2018) [82] in Jimma town, Hailu *et al*. (2015) [17] in Gondar town, and Adzitey *et al*. (2020) [90] in the Tamale Metropolis of Ghana who reported 100.0% sensitivity to Ciprofloxacin. Sensitivity to Nalidixic acid, Sulfamethoxazole-Trimetoprim, Amoxicillin and Kanamycin was also observed in 85.2%, 81.5%, 77.8%, and 70.4% of the isolates, respectively. The sensitivity results of the isolates to Nalidixic acid, Kanamycin, and Amoxicillin were higher than the report of Beyene *et al*. (2016) [78] in Asella town who reported 66.7%, 58.3%, and 33.3% sensitivity to Nalidixic acid, Kanamycin, and Amoxicillin, respectively.

5. Conclusions

The detection of multidrug-resistant *Salmonella* species from foods of bovine origin produced in the study sites indicated that these products were not safe for consumption and may pose a public health problem to the consumers. The bacterial contamination of these products might be due to unhygienic practices during production chain. The multidrug resistance pattern of all *Salmonella* species isolates might be due to extensive and injudicious use of antibiotics both in human and veterinary medicine. Moreover, the expanded illegal open field slaughtering practices, unstandardized slaughtering operations at municipal abattoirs, and unhygienic milk production and handling could prone people to drug-resistant *Salmonella* and other common food-borne bacterial pathogens. Hence, preventive measures are required to reduce the bacterial contamination, and concurrently to improve the wholesomeness and safety of foods of bovine origin. Therefore, regulatory organizations and government officials should establish and monitor control measures and standards for the safe production, transportation, and storage of foods of bovine origin up to consumption; and strategies to improve the judicious antimicrobial use should be developed, implemented and evaluated. Moreover, further studies on serotyping and molecular characterization of *Salmonella* species as well as identification and characterization of their resistant genes should be conducted in the study areas and across the country.

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