

Identification and Antimicrobial Susceptibility Profile of *Salmonella* Isolated from Selected Dairy Farms, Abattoir and Humans at Asella Town, Ethiopia

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Abstract

Salmonella is one of the most common causes of food-borne diarrheal disease in human as well as animals. It is leading causes of acute gastroenteritis when ingested in contaminated foods, including meat and dairy products. Moreover, the emergence of multiple-resistant (MDR) isolates is increasing in human and veterinary medicines. Therefore, this cross-sectional study aims at isolation, identification and antibiogram of *Salmonella* from selected dairy cattle farms, abattoir and in contact humans in both dairy farms and abattoir of Asella, Ethiopia.

We collected 185 samples from abattoir (n=94) and dairy farms (n=91), which were isolated and identified according to ISO-6579, 2002. The overall proportion of *Salmonella* was 6.5% (12/185) (dairy farms n=4, 4.4% and abattoir n=8, 8.5%). Antibiogram of isolated *Salmonella* was also evaluated against ten commonly used antibiotics in both humans and veterinary medicines to treat salmonellosis by using the Kirby Bauer disk diffusion method. All isolates (100%, n=12) were susceptible to ciprofloxacin and gentamycin followed by 91.7%, 75%, 66.7%, 58.3 and 50% of the isolates were susceptible to sulfamethoxazole-trimethoprim, chloramphenicol, kanamycin, nalidixic acid and streptomycin, respectively. However, ceftiofur showed the highest resistance (66.7%) followed by ampicillin and amoxicillin (58.3% each). Moreover, 50% of the isolates were resistant to two or more of the tested antimicrobial agents. The highest MDR was seen on pooled hand swabs from abattoir, resistance to eight antimicrobials (80%, n=8/10) with the combination of ceftiofur, ampicillin, amoxicillin and streptomycin being more frequent.

High proportion of *Salmonella* was isolated from abattoir sample than dairy farms. These isolate developed MDR to commonly prescribed antimicrobial agents in the study area. Hence, strict hygienic management in the farm and abattoir as well as rational use of antimicrobials should be practised to circumvent the further development of antimicrobial resistance.

Keywords: Antibiogram; MDR; Ciprofloxacin; Gentamycin; Ceftiofur; Food safety; Antimicrobial resistance; Abattoir; Antibiotic resistance; Dairy farms; Humans; Isolation; *Salmonella*

Abbreviations:

AMR: Antimicrobial resistance; ARVL: Asella Regional Veterinary Laboratory; CI: Confidence Interval; CLSI: Clinical and Laboratory Standards Institute; ISO: International Organizations for Standardization; MDR: Multidrug-resistance; ml: Milliliter; SPSS: Statistical Product and Service Solution; χ^2 : Chi square; μ g: Microgram.

Introduction

Food borne bacterial diseases are a serious challenge to human and animal health. The epidemiology of these diseases has changed rapidly because of changes in the social environment and the ability of pathogens to adapt to new niches. *Salmonella* is one of the most common causes of food borne diarrheal disease in human and animals. It is leading causes of acute gastroenteritis and an important public health problem worldwide particularly in the developing countries [1].

Salmonella transmits to humans can occur through several routes. These are consumption of contaminated food products (milk, eggs, and meats), direct contact with animals and their environment, 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 [2,3]. Excretion of *Salmonella* with faeces can contaminate water, soil, other animals and feed [4]. Although *Salmonella* primarily intestinal bacteria, due to its ubiquitous nature common in the environment and commonly found in farm effluents, human sewage and in any materials subject to faecal contamination as a result it leads the contamination of milk and meat products to originate either from infected live animals or from cross contamination while during processing [5].

Fluoroquinolones, are effective on the majority of *Salmonella* strains, are usually regarded as the first line treatment of salmonellosis in adult humans [6]. Antimicrobial use in animal production systems has long been suspected to be a cause of the emergence and dissemination of antimicrobial resistant (AMR) *Salmonella*. Using antimicrobial agents for cattle have been implicated as a source of human infection with AMR *Salmonella* through direct contact with livestock and consumption of raw milk, meat and contaminated

material [7]. AMR *Salmonella* are increasing due to the use of antimicrobial agents in food animals at sub therapeutic level or prophylactic doses that may promote growth and markedly increase the human health risks associated with consumption of contaminated milk and meat products [8] through mutation, acquisition of resistance encoding genes [9] and irrational use of antimicrobials in food animals [9,10].

Different studies conducted in Ethiopia revealed fragmented substantial prevalence as well as antimicrobial susceptibility of *Salmonella* in veterinary medicines [8,10-15] and humans [16-18]. However, reports from coinciding study on apparently healthy animals at farm level, carcass at abattoir, and humans involved in working in farms and abattoir is limited. Therefore, the aim of this study were to isolate and identify *Salmonella* from cattle, cattle derivative food (meat and milk) and humans working in the selected dairy farms and abattoir at Asella district as well as compare and evaluate the antibiogram pattern of the isolates from different sources.

Materials and Methods

Study area

The study was conducted from January 2014 to April 2014 in selected dairy cattle farms and municipal abattoir found in Asella.

Asella is at 60591-80491 N latitude and 380411-400441E longitude in central Ethiopia 175 km south east of Addis Ababa. The altitude of the area ranges from 1780-3100 meter above sea level and characterized by mid subtropical temperature ranging from 5°C-28°C. The annual average rainfall is 1200 mm and mostly with clay type of soil and in rare case black soil. The area covers 23674.72 square kilometres and topographically has highland escapement and lowland areas. The high land areas are found centrally and the low lands dominate the periphery of the area [19].

Experimental design

A cross-sectional study was conducted in selected dairy farms located in Asella with a target to supply milk for consumers and in Asella municipal abattoir which is the sole supplier of meat to the town. A total of 185 samples were considered from the abattoir (n=94) and randomly selected cattle of all age and sex groups in the dairy farms (n=91). The sample size was fixed based on representative samples taken from selected dairy cattle farms and municipal abattoir. Samples were collected (Table 1) from four selected dairy cattle farms including small scale farms and six abattoir visits.

Sample source	Sample type	Total sample collected	Salmonella status		χ^2 (P-value)
			Negative	Positive (%)	
Abattoir	Carcass swab	28	27	1 (3.6%)	1.29 (0.256)
	Hanging material swab	7	6	1 (14.3%)	
	Knife swab	6	5	1 (16.7%)	
	Hand swab	7	5	2 (28.6%)	
	lymph node	23	20	3 (13.0%)	
	Faeces	23	23	0 (0.0%)	
	Subtotal	94	86	8 (9.3%)	
Dairy farm	Milk	36	36	0 (0.0%)	
	Tank milk	7	6	1 (14.3%)	
	Faeces from farm	27	26	1 (3.7%)	
	Bucket swab	7	6	1 (14.3%)	
	Hand swab	7	7	0 (0.0%)	
	Tank swab	7	6	1 (14.3%)	
	Subtotal	91	87	4 (4.6%)	
	Total	185	173 (93.5%)	12 (6.5%)	

Table 1: Proportion of *Salmonella* isolated from different samples of dairy farms, abattoir and individuals working in the dairy farms and abattoir.

Specimen collection, transportation and storage

Samples from dairy cows, cattle derivative foods (milk and meat), utensils and personnel working in the farms and abattoir were aseptically collected directly from randomly selected apparently

healthy dairy cattle in the farm and beef cattle in Asella municipal abattoir. Faecal samples were collected directly from the rectum and put into 50 ml containing universal screwed capped bottle and approximately 10 ml of milk was collected aseptically from all teats in a sterile test tube. A pooled swab of carcass, hanging material, lymph

node, hand, tank and bucket was collected by using a sterile wooden cotton swab on the surface of material and insert in the 10 ml test tube that contains sterile buffered peptone water used as a pre enrichment media for 24 hrs at 37°C. Then, within 24 hours, the samples were transported using icebox containing ice bag and analysed at the Asella regional veterinary laboratory (ARVL).

Bacterial culture

The isolation and identification of *Salmonella* from faeces, lymph node, hanging material, knife swab, hand swab, milk and meat was performed at the ARVL by using techniques recommended by International Organizations for Standardization [20]. It involves three steps, 5 gm of faecal sample or 5 ml of milk was pre-enriched with 45 ml of BPW at a ratio of 1:9 and swabs taken from abattoir and farm such as hanging material, knife, hand, bucket and tank was pre-enriched with 10 ml BPW and incubated for 24 hrs at 37°C. One ml of the pre-enriched culture was transferred to 10 ml of Selenite F Broth (SFB) tube and another 0.1 ml portion was transferred to 10 ml of Rappaport Vassiliadis Soy Broth (RVSB) and incubated at 37°C for 24 hrs and 48 hrs, respectively. Finally one loop of broth culture from the inoculated and incubated SFB and RVSB sample was inoculated and incubated on to Xylose Lysine Deoxycholate (XLD) at 37°C for 48 hrs and *Salmonella* Shigella (S-S) agar at 37°C for 24 hrs. Characteristic *Salmonella* colonies, having a slightly transparent zone of reddish colour and a black centre on XLD media and typical *Salmonella* colonies on S-S agar plate cause the colour of the medium to be colorless or transparent colony with black centre.

When suspected colonies were detected, sub cultivation of 4 *Salmonella* colonies from XLD and S-S agar on to a non-selective nutrient agar media plates for confirmation by using biochemical tests including Triple sugar iron agar (TSI), Indole test, urease test, Simon's citrate test, and Methyl red-Vogues proskeurs (MR-VP) test. Atypical biochemical reaction on TSI i.e., alkaline (red) slant, acidic (yellow) butt, H₂S and gas production, citrate utilization as a carbon source, Indole and urease negative, M-R positive, and V-P negative [21] were performed.

Antibiogram of the isolates

Antibiogram of *Salmonella* isolates was tested against ten different antibiotics, namely amoxicillin (25 µg), ampicillin (10 µg), cefoxitin (30 µg), chloramphenicol (30 µg), gentamycin (10 µg), streptomycin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg) and sulfamethoxazole-trimethoprim (25 µg), all from Oxoid company, England by using Kibry-Bauer disk diffusion method following Clinical and Laboratory Standards Institute guidelines [22].

From each isolate, four biochemically confirmed well-isolated colonies grown on nutrient agar were transferred into tubes containing 5 ml of Tryptone soya broth (Oxoid, England). 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 (Oxoid CM 0337 Basingstoke, England) with in 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.

Following incubation, the diameters of zone of inhibition was recorded to nearest millimetres for each disc used and then classified

as resistant, intermediate, and susceptible according to published interpretive chart of CLSI [22].

Statistical analysis

Data entry and management was done using program Microsoft Office Excel 2010 and then analysed by using SPSS Version 20 computer software. The association between *salmonella* status and sample source and the antibiotic sensitivity pattern of isolated *Salmonella* were compared statistically by using Fisher exact test with significance level defined at the p-value less than 0.05 and 95% CI.

Ethical consideration: The Institutional Review Board of College of Veterinary Medicine and Agriculture, Addis Ababa University ethically approved the study. Moreover, both informed and written consent were obtained from the human subjects. Confidentiality of the human participants, abattoir and the dairy farms were maintained by using unique code.

Results and Discussion

Isolation and identification of Salmonella

Salmonella is considered as an important food borne bacterial pathogens. In this study, of 185 samples collected from selected dairy farms and abattoir, it was found that the proportion of *Salmonella* isolated from dairy farms, apparently healthy slaughtered cattle in municipal abattoir and materials used in the process of food along with personnel's hand swab was 6.5% (n=12). Among the isolates, the relative overall proportion of *Salmonella* at farm level was found to be 4.4% (4/91) and at abattoir 8.5% (8/94). However, there was no statistically significant association between the *Salmonella* status and sample source collected from abattoir and farm ($\chi^2=1.291$, $p=0.256$). The proportion of *Salmonella* isolated in this study is lower than previous studies conducted in Ethiopia 20% in raw milk from Korsa district [23], 10.76% in lactating cows and in contact humans in dairy farms of Addis Ababa [11], 7.2% in slaughtered small ruminants and environment in Modjo export abattoir [24] and 7.1% from apparently healthy slaughtered cattle in Debre Zeit [14]. However, it is higher than the previous study on dairy product in Addis Ababa (1.6%) [25,26] and cheese and milk in Debre Zeit (2.1%) [8].

In the present study, the proportion of *Salmonella* on individual sample of carcass swab was 3.6%, mesenteric lymph nodes (13.0%), and pooled knife swabs (16.7%) and pooled hand swabs (28.6%) from abattoir (Table 1). This study has also shown higher proportional isolation of *Salmonella* than report from knife swab (7.4%) and hand swab (8.9%), similar to that reported from mesenteric lymph node (13.0%), but lower isolation from carcass swab (25.0%) than the work of Teklu and Negussie [24]. The reason could be associated with the hygienic status of the abattoir and cross contamination among the materials used in the slaughtering operation and processing of food. The difference in proportion of *Salmonella* isolation between the present studies from the previous studies at different areas of the country could be associated with different 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 [26]. Environment related risk factors are often related to hygienic and management practice, stocking density, type and amounts of feed, accessible water supplies, infection load in the environment, usage of contaminated utensil, housing type, ventilation, flooded grassing areas, movement of animals, calving

environment, and production facilities in different areas are also plays a role for *Salmonella* occurrence [27]. Additionally, epidemiological patterns of *Salmonella* differ greatly between geographical areas depending on climate, population density, land use, farming practice, food harvesting and processing technologies and consumer habits [28].

In the current study though relatively low proportion of *Salmonella* (6.5%) was isolated and identified compared to previous studies, it might pose a significant health risks to humans and animal species to cause salmonellosis in high-risk groups such as new-borns, infants, and the elderly and immune compromised individuals susceptible to *Salmonella* infections at a lower infective dose than healthy adults are. Therefore, it is a source of *Salmonella* infection through consumption of contaminated dairy products, which is mainly important in Ethiopia in general and Asella in particular, where dairy products are frequently consumed without proper boiling.

Antimicrobial susceptibility testing

Salmonella isolates (n=12) were tested against ten commonly used antimicrobials following CLSI [22] guidelines. The results of antimicrobial susceptibility testing showed that 66.7%, 58.3%, and 41.7% resistance to cefoxitin, amoxicillin/ampicillin and streptomycin, respectively while 100% sensitive was recorded to ciprofloxacin and gentamycin followed by 91.7%, 75.0% and 58.3% sensitive to sulphametoxazole-trimethoprim, chloramphenicol and kanamycin respectively (Table 2).

Antimicrobials tested	Status of antimicrobial agent against the isolates		
	Resistant (%)	Intermediate (%)	Susceptible (%)
Amoxicillin	7 (58.3%)	1 (8.3%)	4 (33.3%)
Ampicillin	7 (58.3%)	4 (33.3%)	1 (8.3%)
Cefoxitin	8 (66.7%)	0 (0.0%)	4 (33.3%)
Chloramphenicol	2 (16.7%)	1 (8.3%)	9 (75.0%)
Ciprofloxacin	0 (0.0%)	0 (0.0%)	12 (100%)
Gentamycin	0 (0.0%)	0 (0.0%)	12 (100%)
Kanamycin	2 (16.7%)	3 (25.0%)	7 (58.3%)
Nalidixic acid	1 (8.3%)	3 (25.0%)	8 (66.7%)
Streptomycin	5 (41.7%)	1 (8.3%)	6 (50.0%)
Sulphametoxazole-Trimethoprim	1 (8.3%)	0 (0.0%)	11 (91.7%)

Table 2: Antimicrobial susceptibility profile of *Salmonella* isolated from dairy cattle, abattoir and humans working in the dairy farms and abattoir.

The result for streptomycin resistance in this study (41.7%) was higher than 13.3% and 25%, which was reported by Addis et al. [11] and Tadesse and Anbessa [23], respectively. However, the finding for ampicillin is slight higher than the findings of other investigators in Ethiopia (50%) by Tesfaw et al. [25] but lower than 100% reported by Addis et al. [11] whereas the findings for amoxicillin is higher than 16.7% reported by Tesfaw et al. [25]. The resistance of chloramphenicol in this study is consistent with 16.7% reported by Tesfaw et al. [25] and

Addis et al. [11], and lower than 25% reported by Tadesse and Anbessa [23]. The effectiveness of gentamycin and ciprofloxacin to isolated *Salmonella* in this study (100%) is similar to the result reported by Tesfaw et al. [23], but higher than 73.3% and 83.3% reported by Addis et al. and 75% and 95% reported by Tadesse and Anbessa [23] for both antimicrobial agents, respectively. This difference might be due to small sample sizes for the data, nature of drug, presence of different strain of the bacteria, development of resistant gene, their low frequency usage for prevention and control of disease in food animals in the study area.

Among the 12 *Salmonella* isolates subjected to the antimicrobial susceptibility testing, the majority of the isolates (83.3%, n=10/12) were resistant to at least one or more drugs tested. The result is in line with different studies conducted in Ethiopia by Dabassa and Bacha [29] Tadesse and Anbessa [23] and Tesfaw et al. [25].

In the present study, 50% of the isolates were resistant to at least three or more types of antimicrobials (MDR) and to single type of antibiotic (16.7%) (Table 3) compared with the work of Tadesse and Anbessa [23] who reported 70% and 30%, Dabassa and Bacha [29] who report 83.3% and 16.3%, and also Tesfaw et al. [25] who reported 50% and 50% for multiple and single antimicrobial resistance, respectively.

Number of AMR	Antimicrobials shown resistance	Number of isolates (%)
0	None	2 (16.7%)
1	AML	2 (16.7%)
	FOX	
2	FOX+S	2 (16.7%)
	AMP+FOX	
3	AML+AMP+FOX	3 (25.0%)
	AML+AMP+FOX	
	AML+AMP+S	
5	AML+AMP+FOX+C+S, AML+AMP+FOX+K+S	2 (16.7%)
6	AML+AMP+FOX+C+K+NA+S+SMT	1 (8.3%)

Table 3: Antimicrobial susceptibility pattern of *Salmonella* isolates from different samples of dairy farms, abattoir and individuals working in the dairy farms and abattoir. AMR: antimicrobial resistance; AML: Amoxicillin; AMP: Ampicillin; FOX: Cefoxitin; C: chloramphenicol; K: Kanamycin; NA: Nalidixic acid; S: Streptomycin; SMT: Sulphametoxazole-trimethoprim.

The highest MDR was seen on polled hand swabs from abattoir for eight antimicrobials with the combination of cefoxitin, ampicillin, amoxicillin and streptomycin being more frequent followed by hanging material and knife swabs with a value of five antimicrobials, respectively (Table 4). The difference in AMR level of *Salmonella* in different areas of the country was related to agent risk factors, which might be virulence, pathogenicity, infectiousness, antibiotic resistance, and host specificity mostly determined by the genetic composition of *Salmonella* strain [27]; and other possible causes could be increasing rate of non-rational use of antibiotics in the dairy farms, frequent usage both in livestock and public health, use of counterfeit drugs in

animal husbandry [30] self-medication due to easy access to antibiotics without prescription in public health sector and administration of sub therapeutic dose of antimicrobials to livestock for prophylactic or nutritional purpose in food animals [31,32].

Name of antimicrobial agent	Level	Number of Salmonella isolated and percent of their susceptibility for different antimicrobial agents								
		BS(1)	CS(1)	FF(1)	HS(2)	HM(1)	KS(1)	LN(3)	TM(1)	TS(1)
Ampicillin	S	0	100	0	0	0	0	0	0	0
	I	100	0	100	0	0	0	66.7	0	0
	R	0	0	0	100	100	100	33.3	100	100
Amoxicillin	S	100	0	0	0	0	0	66.7	100	0
	I	0	0	100	0	0	0	0	0	0
	R	0	100	0	100	100	100	33.3	0	100
Streptomycin	S	0	100	100	50	0	0	66.7	0	100
	I	0	0	0	0	0	0	0	100	0
	R	100	0	0	50	100	100	33.3	0	0
Kanamycin	S	100	100	100	0	0	100	100	0	0
	I	0	0	0	50	0	0	0	100	100
	R	0	0	0	50	100	0	0	0	0
Nalidixic acid	S	100	100	100	50	0	100	66.7	100	0
	I	0	0	0	0	100	0	33.3	0	100
	R	0	0	0	50	0	0	0	0	0
Ciprofloxacin	S	100	100	100	100	100	100	100	100	100
	I	0	0	0	0	0	0	0	0	0
	R	0	0	0	0	0	0	0	0	0
Chloramphenicol	S	100	100	100	50	100	0	66.7	100	100
	I	0	0	0	0	0	0	33.3	0	0
	R	0	0	0	50	0	100	0	0	0
Cefoxitin	S	0	100	0	0	0	0	100	0	0
	I	0	0	0	0	0	0	0	0	0
	R	100	0	100	100	100	100	0	100	100
Gentamycin	S	100	100	100	100	100	100	100	100	100
	I	0	0	0	0	0	0	0	0	0
	R	0	0	0	0	0	0	0	0	0
Sulfamethoxazole-Trimethoprim	S	100	100	100	50	100	100	100	100	100
	I	0	0	0	0	0	0	0	0	0
	R	0	0	0	50	0	0	0	0	0
MDRa	N	100	100	100	0	0	0	66.7	100	0

	Y	0	0	0	100	100	100	33.3	0	100
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Table 4: Antimicrobial resistance level of *Salmonella* isolated from different samples of dairy farms, abattoir and individuals working in the dairy farms and abattoir. BS: Bucket swab; CS: Carcass swab; FF: Faeces from farm; HS: Polled hand swab from abattoir; KS: Polled Knife swab; LN: Lymph node; TM: Tank milk; TS: Tank swab; HM: Polled hanging material; S: S Susceptible; I: Intermediate; R: Resistant; MDR: Multi-drug resistance; a: resistant to three or more of the tested antimicrobial agent.

In general, antimicrobial use is a key driver of resistance development, which is either over use for minor infectious, misuse due to lack of access to appropriate treatment and underuse due to inadequate dosing, poor adherence or substandard antimicrobial and lack of financial support to complete treatment course. The present study indicated importance of cattle products (milk and meat) and materials used for processing of these products as potential source of *Salmonella* infection.

Conclusions

Salmonella was isolated from cattle, cattle derivative food and in contact humans in dairy farms and abattoir, which are a potential source of AMR *Salmonella* infection. The overall prevalence of *Salmonella* was 6.5%, where the prevalence in selected dairy farms and municipal abattoir was 4.4% and 8.5%, respectively. Ciprofloxacin and gentamycin are the most effective antibiotics whereas cefoxitin, ampicillin and amoxicillin showed the highest resistance. Half of the tested *Salmonella* isolates was resistant to three or more of the tested antimicrobial agents that are commonly used in the veterinary and human medicines. This might limit therapeutic choice to manage salmonellosis and other bacterial diseases both in animal and human health care. Therefore, further detailed studies should be conducted to describe the common *Salmonella* serovars isolated from animals and humans in the study area and molecular characterization of the isolates resistant genes to identify the mechanism of AMR development.

Competing Interests

None of the authors has any competing interests.

Authors' Contributions

TB participated in research coordination, study design, data analysis, antibiogram, and manuscript drafting and final revision. HY participated in sample collection, bacterial culture and identification, antibiogram and drafting manuscript. BC participated in study supervision, bacterial identification and antibiogram. RD conceived the research idea and participated in its design, coordination and data analysis. FA, AF, DA, BM coordinated and supervised the study, provided valuable information on the subject of and the design of the study. All authors agreed with the results and conclusions; and read and approved the final manuscript.

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