

## Full Length Research Paper

# Distribution and antimicrobial resistance of *Salmonella* serotypes in minced beef, calves and humans in Bishoftu and Addis Ababa, Ethiopia

Dagnachew, D.

College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia.

Received 7 December, 2016; Accepted 20 February, 2017

This study was conducted to determine the prevalence and antimicrobial profile of *Salmonella* in 102 beef, 384 stool and 107 calf faecal samples. The beef samples were collected from 34 randomly selected supermarkets in Addis Ababa, stool samples were collected from Bishoftu General Hospital and calf faecal samples were from randomly selected dairy farms in Bishoftu. Of the total 102 minced beef, 384 stool and 107 faecal samples examined, 9.8, 3.4 and 1.9%, respectively, were positive for *Salmonella*. Twenty-five *Salmonella* isolates comprising of 14 different serotypes were identified. Among the different serotypes, *S. Typhimurium* was predominant (28%) followed by *S. Uganda* (20%) and *S. Bovismorbificans* (8%). The other serotypes identified were *S. Anatum*, *S. Blockley*, *S. Braenderup*, *S. Enteritidis*, *S. Hadar*, *S. Havana*, *S. Livingstone*, *S. Mikawasima*, *S. Muenchen*, *S. Saintpaul* and *S. Typhimurium* var. *Copenhagen* totally comprising 44%. *Salmonella* Mikawasima was reported for the first time in Ethiopia. Assay of antimicrobial resistance revealed that 20% of the isolates were resistant to three or more of the 24 antimicrobials checked. Resistance to 15 antimicrobials was recognized. The most common resistance was to nitrofurantion, streptomycin and tetracycline. Most of the antimicrobial resistant *Salmonella* isolates were from the meat samples. Result of the present study indicate that *Salmonella* isolates are diverse in serotype with significant antimicrobial resistance in the samples tested which could be potential sources of drug resistant *Salmonella* infections.

**Key words:** Addis Ababa, Bishoftu, calf, Ethiopia, human, minced beef, *Salmonella*.

## INTRODUCTION

Domestic animals harbour *Salmonella* in their gastrointestinal tracts and *Salmonellae* are often excreted in faeces by healthy animals with no apparent signs of illness (Loneragan et al., 2012). *Salmonella* frequently contaminates raw foods of animal origin through faecal contact during production and slaughter. Humans generally become infected by eating undercooked or

contaminated food (Majowicz et al., 2010) and beef is often contaminated with *Salmonella* (Guo et al., 2011). Most of the time, invasive non typhoid salmonellosis in human is related to *S. Typhimurium* and *S. Enteritidis* (Reddy et al., 2010). As chopping of meat facilitates additional microbes to adapt, minced beef samples are frequently contaminated with high number of microbes.

E-mail: dagndem@gmail.com.

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

*Salmonella* is often present in minced beef in large amount (Tegegne and Ashenafi, 1998).

Antibiotics are usually applied for the control of diseases in humans and animals. As a result of antibiotic use in food animals, however, drug-resistant pathogens are increasing (Alexander et al., 2009) and this limits therapeutic options both in veterinary and public health practices. Sub-therapeutic and/or prophylactic doses and indiscriminate use of antibiotics in veterinary medicine result in on-farm selection of resistant *Salmonella* which may then pass to humans (Hoelzer et al., 2011; Pui et al., 2011).

Earlier investigations in Ethiopia have demonstrated the presence of *Salmonella* in humans, animals and animal products (Alemayehu et al., 2003; Mache, 2002). *Salmonella* Mishmarhaemek was the predominant isolates in cattle (Tadesse and Tessema, 2014). *Salmonella* Concord, *S. Typhi*, *S. Typhimurium* and *S. Paratyphi* were the dominant serotypes isolated from human and *S. Concord* was reported to be the most common serotype to be resistant to third generation cephalosporins (Tadesse, 2014). *Salmonella* Dublin was the most frequent serotypes isolated from beef (Tadesse and Gebremedhin, 2015). Similarly, other studies in Ethiopia have demonstrated the presence of drug resistance among other *Salmonella* isolates (Haimanot et al., 2010; Beyene et al., 2011; Reda et al., 2011) and *S. Kentucky* was the most frequently reported serotype resistant to ciprofloxacin in animal (Molla et al., 2006; Aragaw et al., 2007).

Real-time investigations on serotype diversity and antimicrobial resistance profile of *Salmonella* in animals, animal products and humans give full understanding of the disease. Therefore, the aim of this study was to determine the prevalence, distribution and antimicrobial resistance profile of *Salmonella* serotypes in calves, minced beef samples and humans in some areas of Bishoftu and Addis Ababa.

## MATERIALS AND METHODS

### Study area and study design

A cross-sectional survey of *Salmonella* in minced beef originating from Addis Ababa supermarkets, calf faeces from Bishoftu commercial and smallholder dairy farms and human stool samples from out-patients of Bishoftu General Hospital was undertaken from October, 2010 to May, 2010. The study involved a total of 593 samples consisting of 102 minced beef, 107 calf faecal samples and 384 human stool samples.

### Sample collection

The meat samples were purchased randomly once a week as sold for consumers (usually in a refrigerated display cases at 4°C) from 34 randomly selected supermarkets. The faecal samples were collected from all calves under 6 months of age from randomly selected commercial and smallholder dairy farms in Bishoftu. The

stool samples were collected randomly from out-patients in collaboration with the medical personnel in the hospital. Samples were identified by sample number, date of sampling, source and sample type. Minced beef samples were collected in a plastic material with which meat was distributed to the consumer or pre-packed in polyethylene bags. The faecal and stool samples were collected using sterile universal culture bottles. Samples were then taken to the Microbiology Laboratory of the College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu using icebox and kept chilled until microbiological analysis was done.

### Isolation and identification

*Salmonella* isolation and identification was carried out in line with the guidelines of the International Organization for Standardization (ISO, 2002) and Quinn et al. (1999), and steps that include primary enrichment in non-selective liquid medium (pre-enrichment), secondary enrichment in selective liquid media, plating out on selective and non-selective media and final confirmation by biochemical and serological characterization were employed.

### Primary enrichment in non-selective liquid medium (pre-enrichment)

The chilled samples were left for 3 to 5 h at 20 to 22°C before being processed. Twenty-five grams of minced beef, 10 to 25 g of faecal samples and 4 to 5 g of stool samples were added to buffered peptone water (BPW) (OXOID, Hampshire, England) in 1:9 ratio (1 gram of sample to 9 ml of BPW). The mixture was homogenized using a laboratory blender at high speed for 2 min. The enrichments were then incubated aerobically at 37°C for 18 to 24 h.

### Secondary enrichment in selective liquid media

For this purpose, Rappaport-Vassiliadis magnesium chloride/malachite green (RV) (OXOID, Hampshire, England) and selenite cystine (SC) (DIFCO, Becton, Dickinson and Company, USA) broth media were used. From the incubated pre-enrichment culture, 0.1 ml (aliquot) was taken and mixed with 10 ml RV broth and was incubated aerobically at 42°C for 18 to 24 h. Another 1 ml from pre-enrichment culture was mixed with 10 ml SC broth and incubated aerobically at 37°C for 18 to 24 h.

### Plating out and identification

Plating out was done on brilliant green-phenol red-lactose-sucrose (BPLS) agar (MERCK, Darmstadt, Germany) and xylose lysine desoxycholate (XLD) agar (MERCK, Darmstadt, Germany) plates. A loopful from each of the two enrichment broth cultures was streaked onto the two plating out media. The plates are then incubated aerobically at 37°C for 18 to 24 h. Then the plates were examined for the presence of *Salmonella* colonies. Presumptive *Salmonella* colonies with characteristic appearance on both solid media were then streaked onto Rambach agar (MERCK, Darmstadt, Germany) and were incubated aerobically at 37°C for 24 h. Characteristic colonies for *Salmonella*, which appear red on Rambach agar, were then transferred onto nutrient agar (MERCK, Darmstadt, Germany) and incubated aerobically at 37°C for 24 h.

### Biochemical characterization

Colonies suspected to be *Salmonella* were further tested

**Table 1.** List of antimicrobials used and their concentrations.

Antimicrobial	Abbreviations	Breakpoints and concentrations <sup>a</sup>	
		Susceptible at $\leq \mu\text{g/ml}$	Resistant at $\geq \mu\text{g/ml}$
Amikacin	AMK	16	ND <sup>b</sup>
Ampicillin	AMP	ND	32
Amoxicillin/clavulanic acid	AMC	ND	64/16 <sup>c</sup>
Apramycin	APR <sup>d</sup>	ND	32 <sup>e</sup>
Carbadox	CRB <sup>d</sup>	ND	30 <sup>f</sup>
Cephalothin	CEF	ND	32
Ceftriaxone	CRO	8	ND
Ceftiofur	CTF	ND	8
Cefoxitin	FOX	ND	32
Chloramphenicol	CHL	ND	32
Ciprofloxacin	CIP	0.125 <sup>g</sup>	ND
Florfenicol	FLO <sup>d</sup>	ND	16 <sup>h</sup>
Gentamycin	GEN	ND	16
Kanamycin	KAN	ND	64
Nalidixic acid	NAL	ND	32
Neomycin	NEO <sup>d</sup>	ND	16 <sup>e</sup>
Nitrofurantoin	NIT	ND	64 <sup>i</sup>
Spectinomycin	SPT <sup>d</sup>	ND	64 <sup>e</sup>
Streptomycin	STR <sup>d</sup>	ND	32 <sup>e</sup>
Sulfisoxazole	SUL	ND	512
Sulfamethoxazole/trimethoprim	SXT	ND	76/4
Tetracycline	TET	ND	16
Tobramycin	TOB	ND	8
Trimethoprim	TMP	ND	16

<sup>a</sup>The breakpoint concentrations to determine susceptible, intermediate and/or resistance were those specified by the NCCLS standards M31-A and M100-S12. <sup>b</sup>ND, not done. <sup>c</sup>The strains were considered resistant when growing on agar plates with amoxicillin/clavulanic acid at 64/16  $\mu\text{g/ml}$ . <sup>d</sup>There are no interpretative standards specified by the NCCLS standards M31-A and M100-S12 for apramycin, carbadox, florfenicol, neomycin, spectinomycin and streptomycin. <sup>e</sup>Strains were considered to be resistant to apramycin, neomycin, spectinomycin and streptomycin at 32, 16, 64, and 32  $\mu\text{g/ml}$ , respectively. <sup>f</sup>The strains were considered to be resistant to carbadox, a veterinary growth promoter for pigs, at 30  $\mu\text{g/ml}$ . <sup>g</sup>A 0.125  $\mu\text{g/ml}$  of ciprofloxacin concentration determines reduced sensitivity to ciprofloxacin. <sup>h</sup>Strains were considered to be resistant to florfenicol at the level of 16  $\mu\text{g/ml}$ . <sup>i</sup>Strains were considered to be resistant to nitrofurantoin at 64  $\mu\text{g/ml}$ ; human urinary tract isolates are considered to be resistant to nitrofurantoin at 128  $\mu\text{g/ml}$ .

biochemically using triple sugar iron (TSI) agar slants (BBL, USA), lysine decarboxylase test using lysine decarboxylase broth (DIFCO, Becton, Dickinson and Company, USA), urease test using urea broth (MERCK, Darmstadt, Germany) and citrate utilization test using Simmon's citrate agar (DIFCO, USA). The TSI agar was inoculated; lysine decarboxylase test, urease test and citrate utilization test were conducted according to Quinn et al. (1999).

### Serological characterization

Colonies that exhibited typical reactions for the battery of the biochemical tests were further confirmed by agglutination test by *Salmonella* polyvalent O antiserum (DIFCO, Becton, Dickinson and Company, USA) for the presence of *Salmonella* antigen. Before slide agglutination was performed, a loopful of colonies were suspended in a drop of normal saline on a microscope slide and examined for auto-agglutination. Then, a drop of *Salmonella* polyvalent O antiserum was placed on a clean slide, to which a loopful of colony from nutrient agar was transferred, mixed and rocked for one minute and examined for agglutination.

Finally, *Salmonella* isolates were sent to Salmonellosis

Reference Laboratory, Ontario, Canada, for serotyping, phage typing and antimicrobial resistance investigation.

### Serotyping and phage typing

The somatic (O) and flagellar (H) antigens of *Salmonella* isolates were determined using slide agglutination test (Ewing, 1986) and micro-technique method (Shipp and Rowe, 1980), respectively. The antigenic formula of Le Minor and Popoff (1997) was used to name the serotypes. Phage typing of *Salmonella* Enteritidis was performed according to Ward et al. (1987) using typing phages obtained from the International Centre for Enteric Phage Typing, Central Public Health Laboratory, Colindale, UK.

### Antimicrobial susceptibility test

All *Salmonella* isolates were tested for susceptibility against 24 antimicrobial agents in accordance with the National Committee for Clinical Laboratory Standards (NCCLS, 1999) guidelines. The list and concentrations of antimicrobials used were shown on Table 1.

**Table 2.** The prevalence of *Salmonella* in faecal, stool and minced beef samples.

Sample type	Number of samples examined	Number (%) of samples positive for <i>Salmonella</i>
Calf faeces	107	2 (1.9)
Human stool	384	13 (3.4)
Minced beef	102	10 (9.8)
<b>Total</b>	<b>593</b>	<b>25 (4.2)</b>

**Table 3.** *Salmonella* serotypes isolated by source.

Source	Serotype (number)	Antigenic structure	Phage type
Faeces (n=107)	<i>S. Typhimurium</i> (2)	4,5:i:2	
	<i>S. Typhimurium</i> (5)	4,5:i:2	
Stool (n=384)	<i>S. Typhimurium</i> var. Copenhagen (1)	4:i:2	
	<i>S. Anatum</i> (1)	10:eh:6	
	<i>S. Havana</i> (1)	23:fg:-	
	<i>S. Enteritidis</i> (1)	9,12:gm:-	911
	<i>S. Muenchen</i> (1)	6,8:d:2	
	<i>S. Mikawasima</i> (1)	6,7:y:z15	
	<i>S. Saintpaul</i> (1)	4:eh:2	
	<i>S. Uganda</i> (1)	10:1,z13:5	
	<i>S. Uganda</i> (4)	10:1,z13:5	
	<i>S. Bovismorbificans</i> (2)	6,8:r:5	
Minced beef (n=102)	<i>S. Braenderup</i> (1)	6,7:ehz15	
	<i>S. Livingstone</i> (1)	6,7:d:1,w	
	<i>S. Hadar</i> (1)	6,8:z10:x	
	<i>S. Blockley</i> (1)	6,8:k:5	

In this study, *Salmonella* isolate was considered resistant if it was resistant to at least one antimicrobial drug and multiple resistant if it was resistant to two or more antimicrobial drugs tested.

Moreover, isolates with intermediate resistance were considered susceptible to that antimicrobial. Standard and reference strains, which include *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa*, ATCC 27853, *Staphylococcus aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212 were used.

## RESULTS

### Prevalence and distribution

Of the total 593 faecal, stool and minced beef samples examined, 25 were found contaminated with *Salmonella* (Table 2).

### Serotyping

Twenty-five *Salmonella* positive samples representing 14 different serotypes were identified from 593 samples examined. The most common serotypes identified in this

study were *S. typhimurium* (28%) and *S. Uganda* (20%) followed by *S. bovis morbificans* (8%). *Salmonella* serotypes less commonly isolated include *S. anatum*, *S. blockley*, *S. braenderup*, *S. enteritidis*, *S. hadar*, *S. havana*, *S. livingstone*, *S. Mikawasima*, *S. Muenchen*, *S. Saintpaul* and *S. Typhimurium* var. Copenhagen (Table 3).

*S. typhimurium* was the most dominant serotype detected in this study. It was isolated from 2 faecal samples and from 38.5% of the stool samples. However, this serotype was not recovered from minced beef samples. *S. typhimurium* var. Copenhagen, *S. anatum*, *S. Havana*, *S. enteritidis*, *S. muenchen*, *S. mikawasima* and *S. Saintpaul* were detected only in stool samples, whereas *S. bovis morbificans*, *S. braenderup*, *S. Livingstone*, *S. hadar* and *S. Blockley* were detected only from meat samples.

Nine different serotypes from human stool samples and 6 different serotypes from minced beef samples were isolated, whereas *S. Typhimurium* was the only serotype detected from calf faecal samples. Of the *Salmonella* serotypes isolated from human out-patients, *S.*

**Table 4.** Distribution of resistant *Salmonella* serotypes by source.

Source	<i>Salmonella</i> isolates		
	Serotype tested (Number)	Resistant (%)	Resistant serotype
Faeces	S. Typhimurium (2)	-	-
Minced beef	S. Uganda (4)	3 (30)	S. Blockley
	S. Bovismorbificans (2)		S. Braenderup
	S. Braenderup (1)		S. Hadar
	S. Livingstone (1)		
	S. Hadar (1)		
Stool	S. Blockley (1)	2 (15.4)	S. Enteritidis
	S. Typhimurium (5)		S. Typhimurium var. Copenhagen
	S. Typhimurium var. Copenhagen (1)		
	S. Anatum (1)		
	S. Havana (1)		
	S. Enteritidis (1)		
	S. Muenchen (1)		
Total	S. Mikawasima (1)	5 (20)	-
	S. Saintpaul (1)		
	S. Uganda (1)		
	25		

*typhimurium* (38.5%) was dominant. *Salmonella* Uganda was a predominant serotype isolated from minced beef representing 40% of the isolates followed by *S. bovis* (20%). One serotype, *S. mikawasima*, was reported in Ethiopia for the first time. This new finding might underline the consequence of human movement as infection with *S. mikawasima* is increasing in European countries.

#### Antimicrobial resistance of *Salmonella*

Five isolates (20%) belonging to 5 different serotypes were found multidrug resistant (MDR), that is, resistant to 3 to 9 antimicrobials tested. Thirty percent (3/10) and 15.4% (2/13) of *Salmonella* isolates from meat and stool samples, respectively, were found resistant to 3 or more ampicillin, amoxicillin/clavulanic acid, chloramphenicol, florfenicol, nitrofurantoin, streptomycin, spectinomycin, sulfisoxazole and tetracycline. *S. Blockley* was resistant to ciprofloxacin, kanamycin, nalidixic acid, neomycin, nitrofurantoin, streptomycin and tetracycline. *S. Braenderup* was resistant to ampicillin, spectinomycin, streptomycin, sulfisoxazole, sulfamethoxazole/trimethoprim, tetracycline and trimethoprim. *S. Enteritidis* was resistant to ciprofloxacin, nalidixic acid and nitrofurantoin, whereas *S. Hadar* was resistant to nitrofurantoin, streptomycin and tetracycline (Table 6). All isolates belonging to *S. Anatum*, *S. Bovismorbificans*, *S. Havana*, *S. Livingstone*, *S. Mikawasima*, *S. Muenchen*, *S. Saintpaul* and *S.*

of the antimicrobials checked. With regard to sources of the five resistant *Salmonella* isolates, minced beef accounted for 60% (3/5) and human stool accounted 40% (2/5). *Salmonella* isolates from calf faeces were susceptible to all the 24 antimicrobials used (Table 4).

Nine of the 24 (37.5%) antimicrobials used were effective against all *Salmonella* isolates with the exceptions of carbadox, cephalothin, ceftiofur, florfenicol and kanamycin which showed intermediate resistance pattern to the isolates (Table 5).

Among multidrug resistant isolates, resistance to nitrofurantoin, streptomycin and tetracycline was most often observed. Of the multiple resistant serotypes, *S. Typhimurium* var. Copenhagen was predominant (resistant to 9 antimicrobials) followed by *S. Blockley* and *S. Braenderup* each resistant to 7 antimicrobials. *S. Typhimurium* var. Copenhagen was resistant to 7 antimicrobials. *S. Typhimurium* were susceptible to all 24 antimicrobials. Similarly, all isolates of *S. Uganda* were susceptible to all antimicrobials with the exception of one isolate from stool which showed intermediate resistance to cephalothin ceftiofur, florfenicol and kanamycin. *Salmonella* Blockley also showed intermediate resistance to carbadox (Table 6).

#### DISCUSSION

##### Prevalence and distribution of *Salmonella*

*Salmonella* contamination rate of 9.8% in minced beef

**Table 5.** Resistance pattern of *Salmonella* isolates to the tested antimicrobials.

Antimicrobials <sup>a</sup>	Number of resistant <i>Salmonella</i> serotypes by source		
	Faeces	Stool	Minced beef
Ampicillin	-	1	1
Amoxicillin/clavulanic acid	-	1	-
Carbadox	-	-	1*
Cephalothin	-	1*	-
Ceftiofur	-	1*	-
Chloramphenicol	-	1	-
Ciprofloxacin	-	1	1
Florfenicol	-	1*	-
Kanamycin	-	1*	1
Nalidixic acid	-	1	1
Neomycin	-	-	1
Nitrofurantoin	-	2	2
Spectinomycin	-	1	1
Streptomycin	-	1	3
Sulfisoxazole	-	1	1
Sulfamethoxazole/trimethoprim	-	-	1
Tetracycline	-	1	3
Trimethoprim	-	-	1

<sup>a</sup>Resistance to Amikacin, Apramycin, Ceftriaxone, Cefoxitin, Gentamycin and Tobramycin was not observed; \*Intermediate in resistance.

**Table 6.** Multiple resistance pattern of *Salmonella* isolates.

<i>Salmonella</i> serotype	Number of serotypes tested	Multiple resistant	Antimicrobial resistance pattern	Remark
S. Anatum	1	-	-	
S. Blockley	1	1	CIP, KAN, NAL, NEO, NIT, STR, TET, CRB*	
S. Bovismorbificans	2	-	-	
S. Braenderup	1	1	AMP, SPT, STR, SUL, SXT, TET, TMP	
S. Enteritidis	1	1	CIP, NAL, NIT	Phage type 911
S. Hadar	1	1	NIT, STR, TET	
S. Havana	1	-	-	
S. Livingstone	1	-	-	
S. Mikawasima	1	-	-	
S. Muenchen	1	-	-	
S. Saintpaul	1	-	-	
S. Typhimurium	7	-	-	
S. Typhimurium var. Copenhagen	1	1	AMP, AMC, CHL, FLO, NIT, SPT, STR, SUL, TET	
S. Uganda	5	1*	CEF*, CTF*, FLO*, KAN*	
<b>Total</b>	<b>25</b>	<b>5</b>	-	

\* Intermediate in resistance.

was in agreement with the findings of previous study undertaken in Ethiopia (Ashenafi, 1994). This was also consistent with the 8% prevalence of *Salmonella* from minced meat reported from Egypt (WHO, 1988) and 1.8

to 20% report of *Salmonella* from various countries (D'Aoust, 1989). However, the current finding was lower than the 14.4% (Ejeta et al., 2004), 40% (Molla et al., 1999a) and 42% (Tegegne and Ashenafi, 1998)

prevalence rates reported in Ethiopia. The difference could be due to improvements of sanitation in the supermarkets, seasonal influence or variations between sample sizes and number of supermarkets included. Methodology of isolation employed such as variation in media used for isolation, sampling procedures and one versus multiple picks of suspect colonies for confirmation could also contribute to the difference.

The 3.4% *Salmonella* isolation rate from human stool samples was lower than 4.5% (Ashenafi and Gedebo, 1985) and 7.9% (Mache et al., 1997) reported from Ethiopia and 15.3% reported from Culcutta, India (Saha et al., 2001). Samples examined by all the aforementioned studies originated from diarrhoeic out-patients. This and the slightly different methods employed to culture *Salmonella* may have attributed to the differences observed.

In this study, the prevalence of 1.9% *Salmonella* in calf faeces was in agreement with 2% faecal shedding of *Salmonella* reported (Williams et al 1978). However, this finding was significantly lower than 6.7% reported from apparently healthy cattle in Botswana (Miller, 1971). The low estimate in this study might be due to low proportion of *Salmonella* carriers in the study population and the low and intermittent nature of faecal shedding of carrier animals.

*S. typhimurium* was the most dominant serotype isolated from human out-patients in this study and this serotype was the most reported serotype in human salmonellosis in many countries (Fisher, 2004). It is worth mentioning that *S. mikawasima* was isolated for the first time in Ethiopia. Report of *S. mikawasima* for the first time might underline the risk of introduction of salmonellosis as a result of traffic of people, livestock and foodstuffs across national boundaries.

The identification of six different serotypes from ten isolates from minced beef samples is indicative for the potentially widespread presence of *Salmonella* serotypes in cattle and minced beef in Ethiopia. *S. typhimurium* was detected from apparently healthy calves and this would be regarded as a greater concern because of its demonstrable significance both in animal and human health.

All *Salmonella* isolates in this study from human stool samples belong to non-typhi serogroups. This was not consistent with earlier studies undertaken in Addis Ababa indicating *S. Typhi* a predominant serotype isolated (Afeworki, 1985; Messele and Alebachew, 1981). This was so because most of the samples in the aforementioned studies were blood samples.

#### Antimicrobial resistance of *Salmonella*

The current 15.4% multidrug resistant (MDR) *Salmonella* isolates from minced beef was lower than previous reports in Ethiopia (Tibajjuka et al., 2002; Molla et al.,

2004), but much higher than the one reported in USA (Fluckey et al., 2007). The differences could be as a result of the varied serotypes identified in the respective studies and variation of antimicrobial usage in the study population.

Unlike other reports (Molla et al., 1999b; Gebreyohannes et al., 1987), no *S. Typhimurium* isolate in this study was found resistant to any of the antimicrobial drugs tested. This might indicate the survival of some non-resistant *S. Typhimurium* strains circulating in the population.

On the other hand, the 30% (3/10) *Salmonella* resistant isolates from minced beef in this study and absence of resistance in Nyeleti et al. (2000) was contrasting. This is an indication for the emerging of drug resistant *Salmonella* through time.

All *S. Uganda* serotypes from minced beef were susceptible to all of the antimicrobials used. However, one strain of *S. Uganda* from human stool sample showed intermediate resistance to kanamycin, cephalothin, florfenicol and ceftiofur. This might be due to frequent usage of these drugs in public health than in veterinary medicine.

Resistance was not detected against amikacin, apramycin, carbadox, cefoxitin, ceftriaxone, ceftiofur, cephalothin, gentamycin and tobramycin with the exceptions of carbadox, cephalothin, ceftiofur, florfenicol and kanamycin with intermediate resistance. Resistance was low to amoxicillin/clavulanic acid, chloramphenicol, florfenicol, kanamycin, neomycin, sulfamethoxazole/trimethoprim and trimethoprim. The absence or low levels of resistance to the aforementioned antimicrobials were possibly because of their narrow prescriptions in Ethiopia.

#### Conclusion

The present study demonstrated that *Salmonellae* are present in minced beef, calves and humans in Bishoftu and Addis Ababa. In general, the detection of 14 different serotypes from 25 *Salmonella* isolates and the isolation of *Salmonella* Mikawasima for the first time in Ethiopia indicate the occurrence of widespread *Salmonella* serotypes in the country. The finding of 5 multidrug resistant *Salmonella* serotypes in this study signifies how treatment of clinical salmonellosis is becoming difficult both in animals and humans. Moreover, resistance to nitrofurantoin, streptomycin and tetracycline was an indication of their easy admittance and broad practice in livestock and humans. This justifies the need for strict intervention measures to make sure prudent utilization of antimicrobials and monitoring of drug resistance.

#### CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

## ACKNOWLEDGEMENTS

Dr. Bayleyegn Molla Zewde from United States Department of Agriculture, Food Safety and Inspection Services (USDA FSIS), Denver District, Colorado, is acknowledged for his material support and guidance. Serotyping, phage typing and antimicrobial resistance profile of *Salmonella* isolates were kindly performed at Office International des Epizooties (OIE) Reference Laboratory for Salmonellosis, Ontario, Canada and Dr. Catherine Anne Muckle and Dr Cornelius Poppe are acknowledged.

## REFERENCES

- Afeworki GY (1985). *Salmonella* from Ethiopia: Prevalent species and their susceptibility to drugs. *Ethiop. Med. J.* 23(3):97.
- Alemayehu D, Molla B, Muckle A (2003). Prevalence and antimicrobial resistance patterns of *Salmonella* isolates from apparently healthy slaughtered cattle in Ethiopia. *Trop. Anim. Health Prod.* 35(4):309-319.
- Alexander KA, Warnick LD, Wiedmann M (2009). Antimicrobial resistant *Salmonella* in dairy cattle in the United States. *Vet. Res. Com.* 33(3):191-209.
- Aragaw K, Molla B, Muckle A, Cole L, Wilkie E, Poppe C, Hildebrandt G (2007). The characterization of *Salmonella* serotypes isolated from apparently healthy slaughtered pigs at Addis Ababa abattoir, Ethiopia. *Prev. Vet. Med.* 82(3):252-261.
- 82(3):252-261. Ashenafi M (1994). Microbial flora and incidence of some foodborne pathogens on fresh raw beef from butcher's shops in Awassa, Ethiopia. *Bull. Anim. Health Prod. Afr.* 42:273-277.
- Ashenafi M, Gedebou M (1985). *Salmonella* and *Shigella* in adult diarrhoea in Addis Ababa-prevalence and antibiograms. *Trans. Royal Society Trop. Med. Hyg.* 79(5):719-721.
- Beyene G, Nair S, Asrat D, Mengistu Y, Engers H, Wain J (2011). Multidrug resistant *Salmonella* Concord is a major cause of salmonellosis in children in Ethiopia. *J. Infect. Dev. Ctries* 5(01):023-033.
- Ejeta G, Molla B, Alemayehu D, Muckle A (2004). *Salmonella* serotypes isolated from minced meat beef, mutton and pork in Addis Ababa, Ethiopia. *Rev. Med. Vet.* 155:547-551.
- Ewing WH (1986). Serologic identification of *Salmonella*. In: Ewing, W.H. (ed.). *Edwards and Ewing's Identification of Enterobacteriaceae*, 4<sup>th</sup> ed., Elsevier Science Publishing Co. Inc., New York pp. 201-238.
- Fisher IS (2004). Dramatic shift in the epidemiology of *Salmonella* enterica serotype *Enteritidis* phage types in Western Europe, 1998-2003: results from the Enter-net international *Salmonella* database. *Eurosurveillance* 9(11):43-45.
- Fluckey WM, Loneragan WG, Warner R, Brashears MM (2007). Antimicrobial drug resistance of *Salmonella* and *Escherichia coli* isolates from cattle faeces, hides, and carcasses. *J. Food Prot.* 70(3): 551-556.
- Gebre-yohannes A, Mamo K, Wolde H (1987). R-factor mediated multidrug resistance of *Salmonella* Typhimurium isolates. *Ethiop. Med. J.* 25(1):53-54.
- Guo C, Hoekstra RM, Schroeder CM, Pires SM, Ong KL, Hartnett E, Naugle A, Harman J, Bennett P, Cieslak P, Scallan E, Rose B, Holt KG, Kissler B, Mbandi E, Roodsari R, Angulo FJ, Cole D (2011). Application of bayesian techniques to model the burden of human salmonellosis attributable to U.S. food commodities at the point of processing: Adaptation of a Danish model. *Foodborne Pathog. Dis.* 8(4):509-516.
- Haimanot T, Abdissa A, Beyene G, Gebre-Selassie S (2010). Microbial flora and food borne pathogens on minced meat and their susceptibility to antimicrobial agents. *Ethiop. J. Health Sci.* 20(3):134-143.
- Hoelzer K, Switt AIM, Wiedmann M (2011). Animal contact as a source of human non-typhoidal salmonellosis-Review. *Vet. Res.* 42(1), 34.
- International Organization for Standardization (ISO) (2002). *Microbiology of Food and Animal Feeding Stuff-Horizontal Method for the Detection of Salmonella*, 4 Edn. ISO 6579, Geneva, Switzerland.
- Le Minor L, Popoff LY (1997). Antigenic formulas of the *Salmonella* serotypes. WHO Collaborating Center for Reference and Research on *Salmonella*. Pasteur, Institute, Paris, France.
- Loneragan GH, Thomson DU, McCarthy RM, Webb HE, Daniels AE, Edrington TS, Nisbet DJ, Trojan SJ, Rankin SC, Brashears MM (2012). *Salmonella* diversity and burden in cows on and culled m dairy farms in the Texas high plains. *Foodborne Pathog. Dis.* 9(6):549-555.
- Mache A (2002). *Salmonella* serogroups and their antibiotics resistance patterns isolated from diarrheal stools of pediatric outpatient in Jimma Hospital and Jimma Health Centre, South West Ethiopia. *Ethiop. J. Health Sci.* 12(1):37-46.
- Mache A, Mengistu Y, Cowley S (1997). *Salmonella* serogroups identified from adult diarrhoeal out-patients in Addis Ababa, Ethiopia: antibiotic resistance and plasmid profile analysis. *East Afr. Med. J.* 74(3):183-186.
- Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, Jones TF, Fazil A, Hoekstra RM (2010). The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clin. Infect. Dis.* 50(6):882-889.
- Messele G, Alebachew T (1981). Antimicrobial resistance and R-factors of *Salmonella* isolates from Addis Ababa. *Ethiop. Med. J.* 19(3):77-85.
- Miller AS (1971). Salmonellosis in Botswana. I. Incidence in cattle. *J. Hyg.* 69(03):491-496.
- Molla B, Berhanu A, Muckle A, Cole L, Wilkie E, Kleer J, Hildebrandt G (2006). Multidrug resistance and distribution of *Salmonella* serotypes in slaughtered pigs. *J. Vet. Med. B.* 53(1):28-33.
- Molla B, Kleer J, Sinell H (1999a). Occurrence, distribution and level of *Salmonella* in selected food items in Addis Ababa, Ethiopia. *Fleisch Wirtschaft International* 4:37-39.
- Molla B, Kleer J, Sinell HJ (1999b). Antibiotic resistance pattern of foodborne *Salmonella* isolates in Addis Ababa, Ethiopia. *Bein. Münch. Tierärztl. Wochenschr.* 112(2):41-43.
- Molla B, Salah W, Alemayehu D, Mohammed A (2004). Antimicrobial resistance pattern of *Salmonella* serotypes isolated from apparently healthy slaughtered camels (*Camelus dromedarius*) in eastern Ethiopia. *Berl. Münch. Tierärztl. Wschr.* 117:39-45.
- National Committee for Clinical Laboratory Standards (NCCLS) (1999). Performance standards for antimicrobial disc and dilution susceptibility tests for bacteria isolated from animals and humans. Document M31-A, Villanova, PA.
- Nyeleti C, Molla B, Hildebrandt G, Kleer J (2000). The prevalence and distribution of Salmonellae in slaughtered cattle, slaughterhouse personnel and minced beef in Addis Ababa (Ethiopia). *Bull. Anim. Health Prod. Afr.* 48(1):19-24.
- Pui CF, Wong WC, Chai LC, Tunung R, Jeyaletchumi P, Noor Hidayah MS, Ubong A, Farinazleen MG, Cheah YK, Son R (2011). *Salmonella*: a food borne pathogen- review. *Int. Food. Res. J.* 18(2): 465-473
- Quinn PJ, Carter ME, Markey B, Carter GR (1999). *Clinical Veterinary Microbiology*. Mosby International Limited pp. 226-234.
- Reda AA, Seyoum B, Yimam J, Andualem G, Fiseha S, Vandeweerd JM (2011). Antibiotic susceptibility patterns of *Salmonella* and *Shigella* isolates in Harar, Eastern Ethiopia. *J. Infect. Dis. Immun.* 3(8):134-139.
- Reddy EA, Shaw AV, Crump JA (2010). Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. *Lancet Infect. Dis.* 10(6): 417-432.
- Saha MR, Saha D, Dutta P, Mitra U, Bhattacharya SK (2001). Isolation of *Salmonella* enterica serotypes from children with diarrhoea in Calcutta, India. *J. Health Popul. Nutr.* 19:301-305.
- Shipp CR, Rowe B (1980). A mechanized micro-technique for *Salmonella* serotyping. *J. Clin. Pathol.* 33(6):595-597.
- Tadesse G (2014). Prevalence of human Salmonellosis in Ethiopia: a systematic review and meta-analysis. *BMC Infect. Dis.* 14(1): 88.
- Tadesse G, Gebremedhin EZ (2015). Prevalence of *Salmonella* in raw animal products in Ethiopia: a meta-analysis. *BMC Research Notes* 8(1):163.

- Tadesse G, Tessema TS (2014). A meta-analysis of the prevalence of *Salmonella* in food animals in Ethiopia. *BMC Microbiol.* 14(1):270.
- Tegegne M, Ashenafi M (1998). Microbial load and incidence of *Salmonella* spp in 'Kitfo', a traditional Ethiopian spiced minced meat dish. *Ethiop. J. Health Dev.* 12(2):135-140.
- Tibaijuka B, Molla B, Hildebrandt G, Kleer J, Salah W (2002). Antimicrobial resistance to salmonellae isolated from retail raw chicken meat and giblets in Ethiopia. *Bull. Anim. Health Prod. Afr.* 50(2):86-95.
- Ward LR, De Sa JDH, Rowe B (1987). A phage-typing scheme for *Salmonella* Enteritidis. *Epidemiol. Infect.* 99(02):291-294.
- World Health Organization (WHO) (1988). Salmonellosis control: The role of animal and product hygiene. Report of WHO Expert Committee Technical Report Series 774, Geneva Switzerland. <http://apps.who.int/iris/handle/10665/39052>.
- Williams DR, Bellhouse R, Davidson CL (1978). The prevalence of salmonellae in healthy cattle at slaughter. *Vet. Rec.* 103(16):359-360