

Carriage and antimicrobial resistance of non-typhoidal *Salmonella* in cattle slaughtered in Ambo municipality abattoir, West Shewa zone, Oromia, Ethiopia - a point prevalence survey

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Abstract

Cattle are reservoir for non-typhoidal *Salmonella* (NTS) serovars which are important foodborne zoonosis worldwide. A cross-sectional study was undertaken from October, 2015 to May, 2016 to estimate the prevalence and assess antimicrobial susceptibility profiles of NTS isolated from slaughtered cattle following standard microbiological technique. A total of 300 samples (150 mesenteric lymph nodes and 150 feces) from 150 cattle slaughtered at Ambo municipality abattoir were investigated. Standard disc-diffusion method was used for antimicrobial susceptibility testing of the isolates. Chi-square and Fisher's exact tests were used to assess association between NTS prevalence and potential risk factors. An overall NTS prevalence of 8% (95 % confidence interval [CI]: 4.2-13.6%) was found at animal level. At sample level, NTS prevalence of 5.6% (17/300) (95% CI: 3.3-8.9%) was found. Prevalence of NTS in mesenteric lymph nodes and fecal samples was 6.6% and 4.6%, respectively. There was no significant difference in prevalence of NTS with respect to sex, age groups and body condition ($p > 0.05$). However, significantly higher prevalence was seen in animals from Guder market (12.5%) than Ambo market (1.6%) ($p < 0.05$). The highest level of antimicrobial resistance was observed for amoxicillin clavulanic acid (100%) and ampicillin (100%) followed by streptomycin (41.1%). Six of the 17 NTS isolates (35.3%) showed multi-drug resistance (MDR) to three or more antimicrobial classes. In conclusion, high percentage of NTS isolates were MDR posing public health threat to consumers. Improved hygiene, education, prudent use of antimicrobials and further large scale epidemiological studies are suggested.

Keywords: Antimicrobial resistance; Cattle; Ethiopia; Non-typhoidal *Salmonella*; Prevalence.

Introduction

Non-typhoidal *Salmonella* (NTS) are important foodborne pathogens that cause gastroenteritis, bacteremia, and subsequent focal infection. They represent an important human and animal pathogen worldwide (Hoelzer *et al.*, 2011). Food animals serve as reservoirs of NTS and pose serious threat to the public health (Wells *et al.*, 2001; Radostits *et al.*, 2007). The consequence of NTS infection in domestic animals ranges from asymptomatic carrier status to acute fatal septicemia and most satisfactorily described as three syndromes; septicemia, acute enteritis and chronic enteritis (Radostits *et al.*, 2007). Cattle play a paramount role as source of foodborne NTS infection. Direct contact with cattle represents a potential human health risk to acquire NTS infection (Hoelzer *et al.*, 2011). The burden of NTS gastroenteritis was estimated at approximately 93.8 million human cases and 155,000 deaths worldwide, and approximately 2.5 million cases and 4,100 deaths per year in Africa (Majowicz *et al.*, 2010).

Non-typhoidal *Salmonella* is often a concern due to the disease it causes in cattle and the potential to infect human that come in contact with cattle or consume its product (Radostits *et al.*, 2007; Hoelzer *et al.*, 2011). Cattle may harbor NTS in lymph nodes and excrete the organisms when stressed (Hoelzer *et al.*, 2011). Fecal shedding of NTS may be brief, intermittent, or persistent (Acha and Szyfres, 2001) and can increase intra-herd transmission, accidental spread to other herds, environmental contamination and risk of human infection (Wells *et al.*, 2001).

The epidemiology of NTS infection is complex which often makes control of the disease difficult. The epidemiology of NTS infection differ greatly between geographical areas depending on climate, population density, land use, farming practices, food harvesting and processing technologies and consumer habits (Radostits *et al.*, 2007). Moreover, the biology of serovars differs so widely that NTS infections are inevitably complex (Popoff *et al.*, 2000). In humans, in addition to concern about foodborne zoonosis caused by NTS, concern has also been raised about the impact of acquired antimicrobial resistance transferred among *Salmonella* serovars (Dargatz *et al.*, 2003). The spread of multidrug resistant NTS serovars have become a global concern (Tadesse and Tessema, 2014) mainly due to the impact in limiting therapeutic options both in veterinary and public health practices (Dargatz *et al.*, 2003). Studies conducted in Ethiopia have shown a high prevalence as well as a high level of antimicrobial

resistance in NTS isolated from various food animals and humans (Molla *et al.*, 2006; Beyene *et al.*, 2011). A recent meta-analysis of prevalence of NTS in food animals in Ethiopia showed 7.0% prevalence in slaughtered cattle (Tadesse and Tessema, 2014). In Ethiopia, where risk of NTS infection is high and therapeutic options are limited, emergence of NTS serovars of animal origin resistant to first and second line antimicrobial drugs used for management of human salmonellosis is a distressing situation (Tadesse, 2015). Although Ambo and its surrounding districts are very important suppliers of beef cattle to consumers in Ambo town and Addis Ababa, to date there is no adequate information about NTS carriage rate as well as antimicrobial resistance of isolates from food animals. Thus, the objectives of this study were to estimate the prevalence and determine the antimicrobial susceptibility of NTS isolated from cattle in Ambo municipality abattoir, Ethiopia.

Materials and methods

Study area

The study was conducted in cattle slaughtered at Ambo municipality abattoir, West Shewa zone, Oromia, Ethiopia. Ambo town is located in Western Shewa Administrative Zone of Oromia Regional State at about 114 Kms West of the capital city, Addis Ababa. Ambo and its vicinity have many tourist attraction sites. Geographically, Ambo is located at altitude of 1900-2275 meters above sea level and latitude and longitude of 8°59'N and 37°51'E. Its temperature ranges from 19°C to 29°C with average annual temperature of 22°C and annual rainfall of about 900 mm. Currently Ambo municipal abattoir is the only slaughterhouse in Ambo town which tries to satisfy the demands of beef for the growing number of over 100,000 inhabitants. Ambo municipal abattoir has the capacity of slaughtering 136 cattle weekly, 546 cattle monthly and 6555 cattle annually (AMAo, 2015). Stunning, evisceration and managing of visceral organs are performed in the same room of slaughter house. The slaughter house has only one room and has three doors out of which two are outlets. Abattoir workers are about 13 in number. They were organized by micro enterprise to do slaughtering, dressing and distribution of carcasses to butcher shops in the town.

Study animals

The study animals were indigenous zebu cattle (*Bos indicus*) raised under extensive and semi-intensive management systems originating from different

districts of West Shewa, Horo Guduru Wallega and East Wallega zones of Oromia Regional State. Some of the animals were kept for certain days at Ambo before being presented to the abattoir for slaughter. There were also some animals bought and presented to the abattoir for slaughter on the same day. Animals were usually slaughtered without being kept for the recommended hours in the premise prior to killing. The age of the animal was estimated according to Amstuz *et al* (1998) and body condition status of the animals was assessed and ranked as good, medium and poor (Nicholsen and Butterworth, 1986).

Study design, sample size and sampling technique

The study was a cross-sectional study conducted from October 2015 to May 2016. The required sample size was calculated using 7.6% expected prevalence reported from Bahirdar (Muluneh and Kibret, 2015) with 5% level of precision and 95% confidence interval using the formula described by Thrusfield (2005), i.e., $n = Z^2 * p \exp (1-p \exp) / d^2$, where n=required sample size; p exp = expected prevalence and a desired absolute precision (d) of 0.05, Z = 1.96.

Accordingly, the minimum sample size calculated was 108. However, to increase the chance of getting more number of NTS positive animals, total of 150 animals and 300 individual samples (from mesenteric lymph nodes and feces, n=150 each) were examined. A systematic random sampling technique was employed to select the slaughtered animals for the study.

Sample collection and transportation

Immediately after slaughtering, feces (100 g) and mesenteric lymph node samples were collected aseptically in a separate clean plastic bag, put in icebox with ice pack and immediately transported to the laboratory of Department of Veterinary Laboratory Technology of Ambo University for isolation and identification of NTS.

Isolation and identification of NTS

Isolation and identification of NTS was made based on the recommendations of the International Organization for Standardization (ISO) method for the detection of NTS from food (ISO 6579, 2002). Briefly, 25 g of lymph node was minced into fine pieces and placed in a stomacher bag (Stomacher 400®, Seward, England) containing 225 ml buffered peptone water (BPW) (1:9) and homogenized well. Similarly, 25 g of fecal sample was mixed with 225 ml BPW and homog-

enized manually by shaking. Then all homogenized samples were incubated at 37°C for about 16 h, and a portion of the culture (0.1 ml) was transferred to a tube containing 10 ml Rappaport-Vassiliadis broth (a selective enrichment liquid medium) (HiMedia Lab Pvt Ltd, India) and incubated at 42°C for 24±3 h. Similarly, 1 ml of the culture was transferred to a tube containing 10 ml of tetrathionate broth (Pronadisa, Spain) and incubated at 37°C for 24±3 h. A loopful of inoculum from each of the enrichment cultures was then inoculated on the surface of two different plates, viz. xylose lysine deoxycholate (XLD) agar (HiMedia Lab Pvt Ltd, India) and brilliant green agar (Pronadisa, Spain) and then incubated at 37°C for 24±3 h. For confirmation, up to five presumptive NTS colonies both from XLD agar and BGA were selected and streaked onto the surface of pre-dried nutrient agar (HiMedia Lab Pvt Ltd, India) plates and incubated at 37°C for 24±3 h. Colonies from nutrient agar were inoculated into the following biochemical tubes for identification: triple sugar iron (TSI) agar (Pronadisa, Spain), lysine iron agar (Pronadisa, Spain), Simmon's citrate agar (HiMedia Lab Pvt Ltd, India), urea agar (HiMedia Lab Pvt Ltd, India), Sulphide Indole Motility (SIM) medium (Sisco research lab Pvt Ltd, India) and incubated for 24 or 48 h at 37°C.

Colonies producing an alkaline (red) slant with acid (yellow) butt with hydrogen sulphide production (blackening) on TSI, positive for lysine (purple color with gas production), positive for citrate utilization (blue color), negative for urea hydrolysis (no color change) and negative for tryptophan utilization (Indole test) (yellow-brown ring) were considered as NTS (ISO 6579, 2002).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of the isolates was performed by using the disc-diffusion method according to the recommendations of the National Committee for Clinical Laboratory Standards (CLSI, 2002; CLSI, 2012). Four to five well-isolated colonies from nutrient agar plates were transferred into tubes containing 5 ml of nutrient broth (Pronadisa, Spain). The broth culture was incubated at 37°C for 4-6 h until it achieved the 0.5 McFarland turbidity standards. Sterile cotton swab was dipped into the suspension, rotated several times, pressing firmly on the inside wall of the tube above the fluid level to remove excess inoculum and swabbed uniformly over the surface of Muller Hinton agar plate (HiMedia Lab Pvt Ltd, India). The plates were held at room temperature for 15 min to allow drying. The discs were placed at least 15 mm apart and from the edge of the plates to prevent overlapping of the inhibi-

tion zones. The plates were incubated at 37 °C for 24 h. The diameter of the zones of inhibitions were recorded and classified as resistant, intermediate or susceptible according to the interpretive standards of the Clinical Laboratory Standards Institute (CLSI, 2012). Standard strains of *E. coli* ATCC 29522 and ATCC 35218 kindly provided by Ethiopian Public Health Institute were used as quality control organisms for the antimicrobial susceptibility test.

The susceptibilities of the isolates were tested for the following antibiotic discs (HiMedia Lab Pvt Ltd, India): amoxicillin-clavulanic acid (AMC) 30 µg, ampicillin (AMP) 10 µg, ciprofloxacin (CIP) 5µg, chloramphenicol (C) 30 µg, kanamycin (KAN) 30 µg, streptomycin (STR) 10 µg, gentamicin (GEN) 10 µg, cefuroxime (CRX) 30µg, nitrofurantoin (NIT) 300 µg and norfloxacin (NOR) 10 µg. The selection of the drugs was based on the standards of drugs used for the treatment of salmonellosis (CLSI, 2012) and the availability of these drugs during this research work.

Data management and analysis

Microsoft Excel was employed for data entry and data analysis was made with STATA version 11 (Stata Corporation, College Station, TX). Descriptive statistics such as frequency, proportion and percentage were used to summarize the results. Animal level prevalence of NTS was calculated as percentage of NTS positive among total number of animals examined. Sample level prevalence was calculated as number of samples positive for NTS divided by total number of samples examined multiplied by 100. Fisher's exact test was used to assess the association of NTS prevalence with the potential risk factors. A 95% confidence interval (CI) was calculated and a *p*-value of <0.05 was considered significant in all the analyses.

Results

The overall prevalence

The prevalence of NTS was assessed at animal and sample level. At animal level, an overall NTS prevalence of 8% (12 of 150) (95% CI: 4.2%-13.6%) was found. At sample level, NTS prevalence of 5.6% (17 of 300) was found. Five animals (3.3%) contained NTS in both their feces and mesenteric lymph nodes (Table 1).

Table 1. Prevalence of non-typhoidal *Salmonella* (NTS) in cattle slaughtered at Ambo municipality abattoir, Ethiopia

Sample	No.		% Prevalence	95% CI
	Tested	Positive		
Feces	150	7	4.6	1.90-9.38
Mesenteric lymph nodes (ML)	150	10	6.6	3.24 - 11.92
Both Feces and ML	150	5	3.3	1.09 – 7.61
Total	300	17	5.6	3.34 - 8.92

CI = confidence interval

Risk factors

There was no significant difference in prevalence of NTS between male and female animals, between age groups (4 -6 years and ≥ 7 years) and body condition scores (poor, medium and good). Significant difference in the prevalence of NTS was observed between the source markets of animals in that the prevalence was significantly higher ($p = 0.016$) in animals brought from Guder market (12.5 %) than Ambo market (1.6%) (Table 2).

Antimicrobial susceptibility of *Salmonella* isolates

All the 17 NTS isolates were resistant to one or more antimicrobial drugs. Out of 17 NTS isolates subjected to 10 antimicrobial drugs, the highest level of resistance was observed for amoxicillin-clavulanic acid (AMC) (100%) and ampicillin (100%) followed by streptomycin (41.1%). All isolates were susceptible to norfloxacin and chloramphenicol (Table 3).

Table 2. Association between non-typhoidal *Salmonella* (NTS) isolation rate and risk factors in cattle slaughtered at Ambo municipality abattoir, Ethiopia.

Risk factors	Category	No. of animals		p-value
		Tested	Positive (%)	
Sex	Female	22	2 (9.1)	0.690
	Male	128	10 (7.8)	
Age	4-6 years	48	4 (8.3)	1.000
	≥7 years	102	8 (7.8)	
Source	Ambo market	62	1 (1.6)	0.015
	Guder market	88	11 (12.5)	
Body condition score	Poor	35	4 (11.4)	0.611
	Medium	81	5 (6.2)	
	Good	34	3 (8.8)	

Table 3. Antimicrobial susceptibility of non-typhoidal *Salmonella* (NTS) isolated from cattle slaughtered in Ambo municipality abattoir, Ethiopia.

Type of antimicrobial	Antimicrobial concentration (µg)	Number (%) of isolates		
		Resistant	Intermediate	Susceptible
Amoxicillin-clavulanic acid	30	17 (100)	0 (0)	0 (0)
Ampicillin	10	17 (100)	0 (0)	0 (0)
Cefuroxime	30	1 (5.9)	4 (23.5)	12 (70.6)
Chloramphenicol	30	0 (0)	0 (0)	17 (100)
Ciprofloxacin	5	6 (35.2)	7 (41.1)	4 (23.5)
Gentamycin	10	5 (29.4)	2 (11.76)	10 (58.8)
Kanamycin	30	3 (17.6)	9 (52.9)	5 (29.4)
Nitrofurantoin	300	5 (29.4)	7 (41.1)	5 (29.4)
Norfloxacin	10	0 (0)	0(0)	17 (100)
Streptomycin	10	7 (41.1)	6 (35.2)	4 (23.5)

Eleven different antimicrobial resistance patterns were recorded (Table 4). Six of the 17 isolates (35.3%) were MDR to three or more classes of antimicrobials while 9 (52.9%) and 2 isolates (11.8%) were resistant to two classes and one class of antimicrobials respectively.

Table 4. Antimicrobial resistance pattern of NTS isolated from cattle slaughtered in Ambo municipality abattoir, Ethiopia

No. of resistance patterns	Resistance pattern (No. of isolates)	No. of resistant isolate (%)
Two	AMC, AMP (3)	3 (17.6)
Three	AMC, AMP, NIT (4) AMC, AMP, STR (1)	5(29.4)
Four	AMC, AMP, CIP, GEN (1) AMC, AMP, CIP, STR (2) AMC, AMP, GEN, STR (1) AMC, AMP, GEN, KAN (1) AMC, AMP, CIP, NIT (1)	6 (35.3)
Five	AMC, AMP, GEN, KAN, STR (1) AMC, AMP, CRX, CIP, STR (1)	2 (11.8)
Six	AMC, AMP, CIP, GEN, KAN, STR (1)	1 (5.9)

AMC=amoxicillin-clavulanic acid; AMP= ampicillin; CIP= ciproflaxlin; STR= streptomycin; CRX= cefuroxime; NIT= nitrofurantoin; GEN=gentamycin; KAN= kanamycin

Discussion

In this study, the prevalence of NTS in mesenteric lymph nodes and feces from apparently healthy slaughtered cattle was estimated at 6.6% and 4.6%, respectively with the overall prevalence of 8%. The overall prevalence of NTS in the present study is comparable to the pooled prevalence estimate (7.07%) recently reported in a meta-analysis of the prevalence of NTS in food animals in Ethiopia (Tadesse and Tessema, 2014). The present animal level prevalence (8%) is also comparable to the 7.1% animal level prevalence previously reported from slaughtered cattle in Ethiopia (Alemayehu *et al.*, 2003). It is higher than the 2.3% prevalence reported in dairy cattle of central Ethiopia (Egualle *et al.*, 2016). Alemu and Zewde (2012) reported lower prevalence (3.2%) from mesenteric lymph nodes as compared to the present finding (6.6%). However, the current prevalence is slightly lower than the work of Sibhat *et al* (2009) who reported 8% and 6% from mesenteric lymph nodes and caecal content, respectively. Very high prevalence of NTS (54.7%) was reported from cows' feces in South Africa (Igbinsa, 2015). The discrepancies in prevalence between different studies could be due to the difference in climate, season, age of animals, diet, management of animals, population density, degree of exposure of animals to stress factors like transportation and starvation, and actual difference in prevalence of NTS in cattle environments (Radostits *et al.*, 2007).

The detection of NTS in feces and mesenteric lymph nodes of slaughtered cattle is of importance in food safety as this can easily result in contamination of carcasses and edible organs during evisceration and other means of contact. Such carcass contamination with NTS is of special public health significance for a country like Ethiopia, where consumption of raw and under cooked meat is common (Teklu and Niguse, 2011). Moreover, the 8% prevalence at animal level is also of great concern considering the fact that animal and humans in Ethiopia live in close contact (Tadesse and Tessema, 2014) and where there is poor hygiene along the meat production chain and inadequate awareness about salmonellosis among consumers.

There was no significant difference in prevalence of NTS between age groups (4 - 6 and >7 years) and body condition scores (poor, medium and good). However, in agreement with the reports of Eguale *et al* (2016), significant difference in the prevalence of NTS was observed between the origin or market source of the animals (Ambo and Guder markets) ($p=0.016$) with the higher prevalence being observed in cattle brought from the Guder (12.5%, 11/88) than Ambo market (1.6%, 1/62). Guder cattle market is one of the largest in Ethiopia supplying slaughter animals for Addis Ababa. Cattle purchased from Guder originate from distant places like East Wallega and Horro Guduru Wallega zones as well as districts of West Shewa zone - Bakko Tibbe, Challia, Mida Kagn, Ilu Gelan and Toke kutaye. Thus, it is highly likely that these animals had traveled on foot long distance, held in different markets before slaughter and suffered from shortage of feed and water. These stress factors might have made the animals shed NTS in their feces, and acquire and harbor new infections in mesenteric lymph nodes (Radostits *et al.*, 2007). Geographical area has also been reported to have significant contribution towards harboring NTS in cattle (Eguale *et al.*, 2016). In agreement with the reports of Eguale *et al* (2016) there is no significant difference in the rate of isolation of NTS between the sexes of animals.

Several earlier studies from Ethiopia (Alemayehu *et al.*, 2003; Molla *et al.*, 2004; Sibhat *et al.*, 2009; Addis *et al.*, 2011; Alemu and Zewde, 2012; Eguale *et al.*, 2016) and elsewhere (Wells *et al.*, 2001; Dargatz *et al.*, 2003; Akoachere *et al.*, 2009; Igbiosa, 2015) have demonstrated that antimicrobial resistant NTS could be isolated from feces and lymph nodes of cattle. In the current study, of the total NTS isolates, 35.3% (6/17) were found to be resistant to three or more antimicrobials. This is higher compared to some previous studies in Ethiopia (Alemu and Zewde, 2012; Ejo *et al.*, 2016). Eguale *et al* (2016) reported that isolates of NTS from cattle feces were resistant to antimicrobials including

streptomycin, nitrofurantoin, and ciprofloxacin. Addis *et al* (2011) also indicated resistance of NTS isolates to commonly used antimicrobials including ampicillin, streptomycin, nitrofurantoin and kanamycin, with resistance rate of 100%, 66.7%, 58.3% and 33.3%, respectively. Similarly, previous reports from Cameroon (Akoachere *et al.*, 2009) and South India (Suresh *et al.*, 2006) indicated 100% resistance to ampicillin while a study from Nigeria (Akinyemia *et al.*, 2005) reported 90% resistance to ampicillin. The 35.2% resistance to ciprofloxacin in the current study is higher than the 9.4% resistance reported from food items previously (Zewdu and Cornelius, 2009) but comparable to the 30% resistance reported from cattle feces in Ethiopia (Eguale *et al.*, 2016).

The results of the current study showed high level of resistance to amoxicillin-clavulanic acid (100%), ampicillin (100%) and streptomycin (41.1%). All NTS isolates were susceptible to norfloxacin and chloramphenicol. This was consistent with the results of Zewdu and Cornelius (2009) and Alemu and Zewde (2012) who reported the susceptibility of all isolates to the chloramphenicol and norfloxacin, respectively. However, susceptibility to chloramphenicol may not suggest that it is good drug in the study area because chloramphenicol may appear active only *in-vitro* for intestinal isolates of NTS species while not active clinically (CLSI, 2014). The high level of resistance to ampicillin is in accordance with the previous studies in Ethiopia (Garedew *et al.*, 2015; Tadesse, 2015) and other parts of the world (88.7%-100%) (Akinyemia *et al.*, 2005; Suresh *et al.*, 2006; Akoachere *et al.*, 2009). However, resistance to amoxicillin-clavulanic acid (100%) in the current study was much higher than the 25% (Zewdu and Cornelius, 2009) and 20.7% (Eguale *et al.*, 2014) reported previously from Ethiopia. This difference could be due to the increasing rate of inappropriate utilization of antimicrobial drugs which applies selection pressure that increases the advantage of maintaining resistance genes in bacteria (Mathew *et al.*, 2007).

The 35.2% resistance to ciprofloxacin (a flouoroquinole) was striking because development of resistance undermines the usage of the drug for human systemic salmonellosis since the drug is among the last options for treatment of complicated non-typhoidal salmonellosis in humans (Hoelzer *et al.*, 2011). The reason for ciprofloxacin resistance in the present study was not well known as this drug is not commonly used in veterinary sectors. However, its usage in public health sector in Ethiopia and elsewhere might have contributed for development of resistance and its introduction into the animals. Nevertheless, there is a need for further investigation to elucidate the situation. Resistance

to nitrofurantoin (29.4%) was relatively lower than the 35.8% (Garedew *et al.*, 2015) and 63.3% (Egualé *et al.*, 2016) previously reported from Ethiopia. Much greater difference was observed in the resistance of streptomycin in the current study (41.1%) as compared to the 86.7% reported previously (Egualé *et al.*, 2016). The increasing level of antimicrobial-resistant NTS might be due to the increased use of antimicrobial agents in food animals which may promote on-farm selection of antimicrobial resistant strains and markedly increase the human health risks associated with consumption of contaminated meat products (Molla *et al.*, 2003; Zewdu and Cornelius, 2009). Among the antimicrobials studied, chloramphenicol and norfloxacin are preferred for therapeutic purpose in the study area followed by cefuroxime. This can be due to proper use of these drugs or unavailability of these drugs in the study area.

The multi-drug resistance (MDR) (35.3%) observed in the current study was higher than the previous MDR reported from Ethiopia ranging from 4% to 28.5% (Molla *et al.*, 2004; Sibhat *et al.*, 2009; Zewdu and Cornelius, 2009; Alemu and Zewde, 2012; Garedew *et al.*, 2015; Ejo *et al.*, 2016). The current MDR is lower than the finding of Addis *et al.* (2011) (83.3%), Egualé *et al.* (2016) (70%), and Alemayehu *et al.* (2003) (52%). The observed MDR in this study might be of great concern in the management of salmonellosis particularly in resource poor settings where new drugs are either absent or unaffordable.

Conclusion

High percentage of NTS isolates are MDR posing great potential threat to consumers. Improved hygienic measures along the meat chain, education of people about food hygiene, prudent use of antimicrobial drugs and further large scale epidemiological studies are suggested.

Acknowledgments

The authors would like to extend their gratitude to Ambo University, Ethiopia, for the financial support, and owners of slaughtered cattle and workers of the Ambo municipality abattoir for their kind cooperation during sample collection.

Conflict of interest

The authors declare that there is no conflict of interest.

References

- Acha, P.N. and Szyfres, B., 2001. Zoonoses and Communicable Diseases Common to Man and Animals. 3rd ed., Washington DC: Pan American Health Organization, Vol.1, Pp. 233- 246.
- Addis, Z., Kebede, N., Worku, Z., Gezahegn, H., Yirsaw, A. and Kassa, T., 2011. Prevalence and antimicrobial resistance of *Salmonella* isolated from lactating cows and in contact humans in dairy farms of Addis Ababa: a cross sectional study. *BMC Infect. Dis.*, 11, 222.
- Akinyemia, K.O., Smith, S.I., Oyefolua, B.A.O. and Coker, A.O., 2005. Multidrug resistance in *Salmonella enterica* serovar Typhi isolated from patients with typhoid fever complications in Lagos, Nigeria. *J. Pub. Hlth.*, 119, 321-327.
- Akoachere, T.K.J., Tanih, F.N., Ndip, M.L. and Ndip, N.R., 2009. Phenotypic characterization of *Salmonella* Typhimurium isolates from food-animals and abattoir drains in Buea, Cameroon. *J. Hlth. Popu. Nutr.*, 27, 1-7.
- Alemayehu, D., Molla, B. and Muckle, A., 2003. Prevalence and antimicrobial resistance of *Salmonella* isolated from apparently healthy slaughtered cattle in Ethiopia. *Trop. Anim. Hlth Prod.*, 35, 309-316.
- Alemu, S. and Zewde, B.M., 2012. Prevalence and antimicrobial resistance profiles of *Salmonella enterica* serovars isolated from slaughtered cattle in Bahir Dar, Ethiopia. *Trop. Anim. Hlth. Prod.*, 44, 595-600.
- AMAO, 2015. Ambo Municipal Abattoir Office annual report. Pp. 1-25.
- Amstutz, G., Miorner, H., Roger, F. and Tibbo, M., 1998. Dental development: In the Merck Veterinary Manual S.E. Aliello Ed.8th, Philadelphia Merck and col.inc, Pp. 131-132.
- Beyene, G., Nair, S., Asrat, D., Mengistu, Y., Engers, H. and Wain, J., 2011. Multi-drug resistant *Salmonella* Concord is a major cause of salmonellosis in children in Ethiopia. *J. Infect. Dev. Ctries.*, 5, 23-33.
- CLSI (Clinical and Laboratory Standard Institute)., 2002. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard- 3rd ed. CLSI document M31-A2, USA, Wayne, Pennsylvania, vol.28 .No. 8.
- CLSI (Clinical and Laboratory Standards Institute)., 2012. Performance for Antimicrobial Disk Susceptibility Tests; Approved the Standard, CLSI Document M02-A11, CLSI, Wayne, Pa, USA, 11th edition, vol. 32, No. 1, Pp. 1-76.

- CLSI (Clinical and Laboratory Standards Institute)., 2014. Performance Standards for antimicrobial Susceptibility Testing. Twenty-Fourth Informational Supplement. CLSI document M100-S24 USA, Wayne, PA 19087, www.clsi.org.
- Dargatz, D.A., Fedorka-Cray, P.J., Ladely, C.A., Koprak, C.A., Ferris, K.E. and Headrick, M.L., 2003. Prevalence and antimicrobial susceptibility of *Salmonella* spp. isolates from US cattle in feedlots in 1999 and 2000. *J. Appl. Microbiol.*, 95, 753-761.
- Egualé, T., Engidawork, E., Gebreyes, W.A., Asrat, D., Alemayehu, H., Medhin, G. *et al.*, 2016. Fecal prevalence, serotype distribution and antimicrobial resistance of Salmonellae in dairy cattle in central Ethiopia. *BMC Microbiol.*, 16, 20.
- Egualé, T., Joanna, M., Molla, B., Aditi, B., Gebreyes, A.W., Engidawork, E. *et al.*, 2014. Association of multicellular behavior and drug resistance in *Salmonella enterica* serovars isolated from animals and humans in Ethiopia. *J. Appl. Microbiol.*, 117, 961–971.
- Ejo, M., Garede, L., Alebachew, Z. and Worku, W., 2016. Prevalence and antimicrobial resistance of *Salmonella* isolated from animal origin food items in Gondar, Ethiopia. *Biomed Res. Int.*, Volume 2016, Article ID 4290506, 8 pages.
- Garede, L., Hagos, Z., Addis, Z., Tesfaye, R. and Zegeye, B., 2015. Prevalence and antimicrobial susceptibility patterns of *Salmonella* isolates in association with hygienic status from butcher shops in Gondar town, Ethiopia. *Antimicro. Resist. Infect. Control.*, 4, 21.
- Hoelzer, K., Switt A.I.M. and Wiedmann, M., 2011. Animal contact as a source of human non-typhoidal Salmonellosis. *Vet. Res.*, 42, 1-27.
- Igbiosa, L.H., 2015. Prevalence and detection of antibiotic-resistant determinant. *Trop. Anim. Hlth Prod.*, 47, 37-43.
- International Organization for Standardization (ISO) 6579., 2002. Microbiology of Food and Animal Feeding Stuff-Horizontal Method for the Detection of *Salmonella*, International Organization for Standardization (ISO), Geneva, Switzerland, 4th edition, Pp. 897-902.
- Majowicz, S.E., Musto, J., Scallan, E., Angulo, F.J., Kirk, M., O'Brien, S.J. *et al.*, 2010. The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clin. Infect. Dis.* 50(6), 882-889.
- Mathew, A.G., Cissell, R. and Liamthong, S., 2007. Antibiotic resistance in bacteria associated with food animals: A United States perspective of livestock production. *Food borne Patho. Dis.*, 4, 115-133.

- Molla, B., Berhanu, A., Muckle, A., Cole, L., Wilkie, E., Kleer, J. and Hildebrandt, G.X. , 2006. Multidrug resistance and distribution of *Salmonella* serovars in slaughtered pigs. *J. Vet. Med. B, Infect. Dis. Vet. Publ. Hlth.*, 53, 28-33.
- Molla, B., Mesfin, A. and Alemayehu, D., 2003. Multiple antimicrobial resistant *Salmonella* serotype isolated from chicken carcass and giblets in Debre-zeit and Addis Ababa, Ethiopia. *Eth. J. Hlth. Dev.*, 17, 131-149.
- Molla, B., Salah, W., Alemayehu, D. and Mohammed, A., 2004. Antimicrobial resistance pattern of *Salmonella* serotypes isolated from apparently healthy slaughtered camels (*Camelus dromedarius*) in eastern Ethiopia. *Berl. MüunchTierärztl. Wschr.*, 117, 39-45.
- Muluneh, G. and Kibret, M., 2015. *Salmonella* spp. and risk factors for the contamination of slaughtered cattle carcass from a slaughter house of Bahir Dar town, Ethiopia. *Asian Pac. J. Trop. Dis.*, 5, 130-135.
- Nicholsen, M.J. and Butterworth, M.J., 1986. A guide to body condition scoring of Zebu cattle, ILCA, Manual, Addis Ababa, Ethiopia, Pp 3728.
- Popoff, M.Y., Bockemühl, J., Brenner, F.W. and Gheesling, L.L., 2001. Supplement 2000 poultry diseases. *J. World's Poult. Sci.*, 61, 574-575.
- Radostits, M.O., Gay, C.C., Hinchcliff, W.K. and Constable, D.P., 2007. Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats. 10th ed. London Elsevier Ltd, Pp. 896-920.
- Sibhat, B., Zewde, B.M., Zerihun, A., Muckle, A., Cole, L., Boerlin, P. *et al.* 2009. *Salmonella* serovars and antimicrobial resistance Profiles in beef cattle, slaughterhouse personnel and slaughterhouse environment in Ethiopia, *Zoonoses Publ. Hlth.*, 58, 102-109.
- Suresh, T., Hatha, A.A., Sreenivasan, D., Sangeetha, N. and Lashmanaperumalsamy, P., 2006. Prevalence and antimicrobial resistance of *Salmonella* Enteritidis and other *Salmonellas* in the eggs and egg-storing trays from retail markets of Coimbatore, South India. *Food Microbiol.*, 23, 294-299.
- Tadesse, G., 2015. A meta-analysis of the proportion of animal *Salmonella* isolates resistant to drugs used against human salmonellosis in Ethiopia, *BMC Infect. Dis.*, 15(1), 84.
- Tadesse, G. and Tessema, T.A., 2014. Meta-analysis of the prevalence of *Salmonella* in food animals in Ethiopia. *BMC Microbiol.*, 14, 270.
- Teklu, A. and Niguse, H., 2011. Assessment of risk factors and prevalence of *Salmonella* in slaughtered small ruminants and environment in an export abattoir, Modjo, Ethiopia. *Am- Eurasian J. Agri. Env. Sci.*, 10, 992-999.

- Thrusfield, M. 2005. Veterinary Epidemiology. 3rd edition, London, Blackwell science Ltd, pp 228-246.
- Wells, S.J., Fedorka-Cray, P.J., Dargatz, D.A., Ferris, K. and Green, A., 2001. Fecal shedding of *Salmonella* spp. by dairy cows on farm and at cull cow markets. *J. Food Prot.*, 64, 3-11.
- Zewdu, E. and Cornelius, P., 2009. Antimicrobial resistance pattern of *Salmonella* serotypes isolated from food items and workers in Addis Ababa, Ethiopia. *Trop. Anim. Hlth. Prod.*, 41, 241-249.