

Full Length Research Paper

Prevalence and antimicrobial susceptibility of *Salmonella* isolates from apparently healthy slaughtered goats at Dire Dawa municipal abattoir, Eastern Ethiopia

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A cross-sectional study was conducted from January to April 2014 on 249 apparently healthy slaughtered goats at the municipal abattoir of Dire Dawa to estimate the prevalence *Salmonella* spp. and determine the antimicrobial susceptibility pattern of the isolates. A total of 249 goat carcass swab samples were collected using a systematic random sampling technique and examined for the presence of *Salmonella* spp. Out of the total of 249 carcass swab samples, 44 (17.7%) were positive for *Salmonella*. Of all the isolates, 41 (93.2%) were multiply antimicrobial resistant and the highest level of resistance was observed for tetracycline (100%), nitrofurans (100%), streptomycin (81.8%) and kanamycin (79.5%). However, all isolates were susceptible to ciprofloxacin. The present study shows high prevalence of *Salmonella* spp. contamination of goat meat and resistance of the pathogen to most antimicrobials except ciprofloxacin. Authors recommended the use of standardized procedures and applications in handling of goat meat in the abattoir and rational use of antimicrobials particularly ciprofloxacin. Furthermore studies should be conducted to identify the potential source of contamination and identification of genes responsible for antimicrobial resistance.

Key words: Abattoir, antimicrobial sensitivity, goat meat, prevalence, *Salmonella*.

INTRODUCTION

Foodborne salmonellosis often occurs following consumption of animal products contaminated with *Salmonella* spp. resulting from infected animals used either in food production or from contamination of the

carcasses or edible viscera during the slaughtering process (Baird-Parker, 1990; Alemayehu et al., 2002; Ejeta et al., 2004). Salmonellosis causes significant morbidity and mortality in both humans and animals and

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has a substantial global socioeconomic impact (Tassios et al., 1997; Hansen-Wester and Hensel, 2001). For instance, annually there are 16 million cases of typhoid fever, 1.3 billion cases of gastroenteritis and 3 million deaths worldwide due to *Salmonella* (Bhunia, 2008).

Antimicrobial-resistant *Salmonella* are increasing due to the use of antimicrobial agents in food producing animals. This may markedly increase the human health risks associated with consumption of meat products contaminated with antimicrobial-resistant *Salmonella*. Animals have been implicated as a source of human infection with antimicrobial resistant *Salmonella* (Zewdu and Cornelius, 2009; Zelalem et al., 2011).

Several studies showed the presence of *Salmonella* in humans, animals, animal food products in many parts of the world (Nyeleti et al., 2000; Muleta and Ashenafi, 2001; Molla et al., 2003; Tibajuka et al., 2003; Woldemariam et al., 2005, Asrat, 2008). There is little published information on the carriage of *Salmonella* in goats, although goat meat has been implicated as a source of *Salmonella* spp. food poisoning (Nabbut and Al-Nakhli, 1982; Chandra et al., 2007; Duffy et al., 2009).

Few studies have been conducted in Ethiopia to isolate *Salmonella* from goats meat and determine the antimicrobial susceptibility of the isolates. These studies focused only in the central part of the country and on export abattoirs (Molla et al., 1999, 2003, 2006; Wassie, 2004; Woldemariam et al., 2005; Akafete and Haileleul, 2011). However, there has been no report regarding the status of antibiotic susceptibility of *Salmonella* spp. from Dire Dawa municipal abattoir. The objectives of this study were to estimate the prevalence of *Salmonella* spp. and antimicrobial susceptibility of *Salmonella* isolates from apparently healthy goats slaughtered at Dire Dawa municipal abattoir.

MATERIALS AND METHODS

Study site

This study was conducted between January, 2014 and April, 2014 at Dire Dawa Administration (DDA) situated at 515 km from Addis Ababa, in the eastern part of Ethiopia. It lies between 90° 27' and 90° 49'N latitudes and between 41° 38' and 42° 19'E longitudes. The rainfall is bimodal and characterized by light rain from February to May and heavy rain from July to September. The mean annual rainfall in the study area varies from 550 to 850 mm. The monthly mean temperature ranges from 14.5 to 34.6°C (DDAEP, 2011).

Study design and population

A cross-sectional study involving microbiological analysis was employed to isolate *Salmonella* spp. The study population comprised apparently health goats slaughtered at the Dire Dawa municipal abattoir.

Sample collection

Two hundred forty nine (249) swab samples were selected using a

systematic randomly technique from apparently healthy goats during slaughtering operations aseptically according to ISO-17604 (2003). The abdomen (flank), thorax (lateral), crutch, breast (lateral), were the sampling sites. Swab samples were taken from each delineated sampling area and all swab samples from a goat were pooled together and kept in a bottle containing buffered peptone water. Samples were kept in boxes containing ice packs and transported to the College of Veterinary Medicine and Agriculture, Addis Ababa University for isolation of *Salmonella* spp.

Salmonella isolation

Salmonella was isolated according to the technique recommended by the International Organization for Standardization (ISO-6579, 2002). The swab samples were pre-enriched in buffered peptone water and incubated at 37°C for 24 h. About 0.1 ml of the pre-enriched sample was transferred into a tube containing 10 ml of Rappaport-Vassiliadis broth and incubated at 42°C for 24 h and 1 ml of the pre-enriched broth was transferred into a tube containing 10 ml of Müller Kauffmann Tetrathionate with novobiocin broth and incubated at 37°C for 24 h. A loop of inoculum from each broth culture was streaked onto Xylose lysine desoxycholate and brilliant green agar plates and incubated at 37°C for 24 h. Five typical or suspected colonies of *Salmonella* were selected from the plates and further streaked onto the surface of pre-dried nutrient agar plates and incubated at 37°C for 24 h. Further biochemical tests using triple sugar iron agar, L-lysine decarboxylation medium, urease and indole production tests were done to isolate *Salmonella* spp.

Antimicrobial susceptibility tests

The 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 (NCCLS, 2002). Four to five well-isolated colonies from nutrient agar plates were transferred into tubes containing 5 ml of tryptone soya broth (Oxoid, England). The broth culture was incubated at 37°C for 4 h until it achieved the 0.5 McFarland turbidity standards. A sterile cotton swab was dipped into the suspension, rotated several times, pressed 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 (Oxoid, England). The plates were kept at room temperature for 30 min to allow drying. Antibiotic discs were placed at least 15 mm apart on the plates and incubated at 37°C for 24 h. The diameter of the zones of inhibitions was compared with recorded diameters of the control organism *E. coli* ATCC 25922 and classified as resistant, intermediate or susceptible according to the interpretive standards of the Clinical Laboratory Standards Institute (CLSI, 2012).

Data management and analysis

The data collected from laboratory investigations were entered into Microsoft Excel and analyzed using SPSS statistical software version 20. Descriptive statistics such as frequency and percentage were used to present the data. P <0.05 was used to see the significant difference among the antimicrobial resistant to *Salmonella* isolates

RESULTS AND DISCUSSION

Out of the total 249 pooled carcass swab samples, 44

Table 1. Antimicrobial susceptibility in salmonella isolates

Type of antimicrobial	Number of isolates		
	Resistant (%)	Intermediate (%)	Susceptible (%)
Ampicillin (AMP) 10 µg	24 (54.5)	2 (4.5)	18 (40.9)
Amoxicillin-clavulanic acid (AMC) 30 µg	20 (45.5)	14 (31.8)	10 (22.7)
Gentamicin (GEN) 10 µg	8 (18.2)	12 (27.3)	24 (54.5)
Kanamycin (KAN) 30 µg	35 (79.5)	6 (13.6)	3 (6.8)
Ciprofloxacin (CIP) 5 µg	-	-	44 (100)
Chloranphenicol (C) 30 µg	20 (45.5)	12 (27.3)	12 (27.3)
Trimethoprim (W) 2 µg	33 (75)	1 (2.3)	10 (22.7)
Sulphonamide (S ₃) 300 µg	19 (43.2)	2 (4.5)	23 (52.3)
Tetracycline (TE) 30 µg	44 (100)	-	-
Nalidixic acid (NA) 30 µg	25 (56.8)	12 (27.3)	7 (15.9)
Ceftriaxone (CRO) 30 µg	10 (22.7)	11 (25)	23 (52.3)
Streptomycin (S) 10 µg	36 (81.8)	5 (11.4)	3 (6.8)
nitrofurantoin (F) 50 µg	44 (100)	-	-

(17.7%) were positive for *Salmonella*. The antimicrobial susceptibility testing of the isolates indicated the highest level of resistance for tetracycline (100%), nitrofurantoin (100%), streptomycin (81.8%) and kanamycin (79.5%). All isolates were susceptible to ciprofloxacin (Table 1). Of all the isolates, 41(93.2%) were multiple antimicrobial resistant (Table 2).

In the present study, out of the total 249 pooled carcass swab samples, 44 (17.7%) were positive for *Salmonella* spp. This percentage is higher in comparison with the reports of Akafete and Haileleul (2011) and Woldemariam et al. (2005) which are 8.3 and 7.5% from export abattoirs, respectively. This difference might be attributed to differences in the hygienic and sanitary practices practiced in the respective abattoirs. The current study was done on municipal abattoir that may have poor sanitation and hygienic standards in comparison with the export abattoirs. Moreover, the high level of contamination with *Salmonella* spp. could be associated with high excretion of *Salmonella* spp. with faeces as source of contamination due to exposure to predisposing factors such as starvation, overcrowding in the market and transportation (Venter et al., 1994). This overall high level of carcass contamination with *Salmonella* spp. is of special public health significance for a country like Ethiopia where consumption of raw and undercooked meat is common.

The current study showed that *Salmonella* spp. isolates were resistant to commonly used antimicrobials including tetracycline, nitrofurans, streptomycin, kanamycin and ampicillin with resistance rate of 100, 100, 81.8, 79.5 and 54.5%, respectively. This result is in agreement with the reports of other researchers from a different area (Akinyemia et al., 2005; Suresh et al., 2006; Akoachere et al., 2009; Zewdu and Cornelius, 2009; Zelalem et al., 2011).

In the present study, ciprofloxacin showed good antimicrobial activity against *Salmonella* spp. isolates. We found that all 44 (100%) isolates were susceptible to ciprofloxacin. This result was comparable to previous reports (Molla et al., 2006; Akinyemia et al., 2005; Zelalem et al., 2011) on isolates of *Salmonella* spp. from different animals and humans. The effectiveness of ciprofloxacin might be attributable to infrequent use of the drug for the treatment of animals and humans in the country indicating the benefit of rational use of the drug (Zelalem et al., 2011).

Resistance to multiple antimicrobials which was observed in the current study (93.2%) was higher than the reports of other studies conducted in Ethiopia. For instance, Alemayehu et al. (2002), Endrias (2004), Molla et al. (2004) and Zelalem et al. (2011) reported 52, 23.5, 44.8 and 83.3%, respectively. In addition, the finding of the present study was higher in comparison with reports on multidrug resistance of *Salmonella* isolated from food of animal sources, animals and humans elsewhere in the world (Stevens et al., 2006; Khaita et al., 2007; Al-Bahry et al., 2007; Elgroud et al., 2009; Fadlalla et al., 2012). This difference could be due to the use of antimicrobial agents in food producing animals and humans at sub-therapeutic level or prophylactic doses and indiscriminate use of antimicrobials (Molla et al., 2003, 2006; Zewdu and Cornelius, 2009). The continuing development of antibiotic resistance may lead to sufficient pressure ultimately to restrict the antibiotics available to the veterinary profession for animal treatment (Gracey et al., 1999). Moreover, this increase antibiotic resistance may lead to public health problems and economic loss in the countries due to loss of exporting meat and animal products and cost of drugs to treat human and animals.

In conclusion, the present study shows high prevalence of *Salmonella* spp. contaminating goat meat and

Table 2. Antimicrobial resistance patterns for *Salmonella* isolates

Number	Antimicrobials (No)	Number (Percentage)
Four	STR, NAL, TET & NIT (1) KAN,W,TET& NIT (2) S3, AMC.TET, NIT (1)	5(11.4%)
Five	STR,KAN,NAL,TMP,AMP,TET& NIT (2) STR,KAN,NAL,TMP,AMC,TET& NIT (1) KAN,S3,NAL,WAMP,TET& NIT (1) STR,CAF,S3,NAL,TMP,TET& NIT (1)	5(16%)
Six	STR,CAF,NAL,TET,GEN,& NIT (1) STR,S3,NAL,AMC,TET& NIT (1)	2(4.5%)
Seven	STR,KAN,NAL,TMP,AMP,TET& NIT (2) STR,KAN,NAL,TMP,AMC,TET & NIT (1) KAN,S3,NAL,TMP,AMP,TET& NIT (1) STR,CAF,S3,NAL,TMP,TET& NIT (1)	5(11.4%)
Eight	STR,CAF,KAN,NAL,TMP,TET,GEN& NIT (2) STR,KAN,NAL,TMP,AMC,AMP,TET&NIT(6) STR,CAF,KAN,S3,CRO,TMP,TET& NIT (1) STR,CAF,KAN,NAL,TMP,AMP,TET&NIT(1)	10(22.7%)
Nine	STR,CAF,KAN,CRO,TMP,AMC,AMP,TET,& NIT (1) STR,CAF,KAN,NAL,TMP,AMC,AMP,TET& NIT (1) STR,CAF,KAN,S3,NAL,TMP,AMC,AMP,TET& NIT (2) STR,KAN,NAL,TMP,AMC,AMP,TET,GEN& NIT (1) STR,CAF,KAN,CRO,NAL,TMP,AMP,TET& NIT (1)	6(13.6%)
Ten	CAF,KAN,S3,CRO,NAL,TMP,AMC,AMP,TET & NIT (2) STR,CAF,KAN,S3,NAL,TMP,AMP,TET,GEN & NIT (1) STR,CAF,KAN,S3,NAL,TMP,AMC,AMP,TET & NIT (1)	4(9.1%)
Eleven	STR,CAF,KAN,S3,NAL,TMP,AMC,AMP,TET,GEN& NIT (2) STR,CAF,KAN,S3,CRO,NAL,TMP,AMC,AMP,TET & NIT (1)	3(6.8%)
Twelve	STR,CAF,KAN,S3,CRO,NAL,TMP,AMC,AMP,TET& NIT(1)	1(2.3%)

AMP = Ampicillin; AMC = amoxicillin-clavulanic acid; GEN = gentamicin; KAN = kanamycin; CIP = ciprofloxacin; CAF = chloranphenicol; TMP = Trimethoprim; S₃ = Sulphonamide; TET = tetracycline; NAL = nalidixic acid; CRO = ceftriaxone; NIT = nitrofurantoin and STR = streptomycin.

resistance of the pathogen to most antimicrobials except ciprofloxacin. Consequently, goat meat provided to the consumers in the city was found to be a potential source of food borne salmonellosis alarming for urgent intervention. Serotyping and phage typing of the isolates are planned. Authors recommended the use of standardized procedures and applications like hazard analysis and critical control point in handling of goat meat in the abattoir to avoid risk of salmonellosis associated with consumption of goat meat contaminated with

Salmonella. Further study ought to be conducted to identify the source of contamination and characterize the molecule of the isolates to identify the resistant genes. Moreover rational use of antimicrobials particularly ciprofloxacin both in veterinary and public health sectors should be exercised.

Conflict of interests

The authors have not declared any conflict of interest.

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