Isolation, Identification, Antimicrobial Susceptibility Test of *Salmonella* on Small Ruminants Meat at Sheikh Abattoir, Somaliland

Abdulkareem Osman Essa

School of Graduate Studies and Research (SGSR), Department of Veterinary Medicine, Gollis University, Somaliland

Abstract: A Case-study involving microbiological analysis and questionnaire survey was conducted between January 2018 and July 2018 in Sheik districts Slaughterhouse, Somaliland. Since there is no report on the status of Salmonella, the study was conducted with the objectives to isolate, identify, estimate the prevalence, to delineate the antimicrobial sensitivity and assess public awareness on Salmonella. A total of 200 carcass swab samples comprising sheep (n=100) and goat meat (n=100) were screened for the presence of Salmonella spp. Out of them, 46 samples (32 sheep and 14 goat meat samples) were found positive for Salmonella spp by culture method with an overall prevalence of 23%. Of all isolates, 44(95.6%) were multiple antimicrobial resistant and highest level of resistance was observed for tetracycline (100%), nitrofurans (100%), Streptomycine (82.6%) and Ampicillin (52.2%). However, all isolates were susceptible to ciprofloxacin. The knowledge, attitude and practices of sheep and goat meat handlers and abattoir workers were found poor. Therefore, sheep and goat meat provided to the city was found less hygienic and not safe for human consumption. Thus, urgent intervention program is essential to minimize the risk associated with consumption of sheep and goat meat contaminated with Salmonella. Finally, the authors recommended that the use of standardized procedures in slaughtering and handling of sheep and goat meat, provision of training on best practice of handling of meat for handlers and raising the level of awareness of people.

Keywords: Isolation, identification, antimicrobial, susceptibility, salmonella, ruminants, meat, Sheikh, abattoir, Somaliland.

I. INTRODUCTION

Infectious microbial diseases constitute a major cause of death in many parts of the world, particularly in developing countries and among them *Salmonella* have been identified as a leading cause of food borne illness in humans and animals resulting in significant morbidity and mortality (Akkina *et al.*, 1999).

Food safety remains a critical issue with outbreaks of foodborne illness resulting in substantial costs to individuals, the food industry and the economy (Kaferstein *et al.*, 1997). Despite advances in food science and technology, foodborne diseases remain one of the major public health and economic problems all over the world (WHO, 1995 and Legnani *et al.*, 2004). The risk of foodborne illness has increased markedly over the last 20 years, with nearly a quarter of the population at higher risk for illness (CDC, 2003; 2004). For instance in the United States, 76 million people get sick, 325,000 hospitalizations, 5,000 Americans die each year from foodborne illness and 2,366,000 cases, 21,138 hospitalizations and 718 deaths in England and Wales (Mead *et al.*, 1999 and Adak *et al.*, 2002). There are about 5.4 million cases of foodborne disease in Australia each year (OzFoodNet, 2006). Hence, trends in foodborne illness in the developed countries indicate that the incidence of foodborne illness is increasing, and that it is likely to remain a threat to public health well into this century (Crerar *et al.*, 1996).

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There are many and varied sources of organisms causing food poisoning. Most cases of food poisoning are caused by bacteria which arise from animal, human or environmental sources (Gracey *et al.*, 1999). Contaminated raw meat is one of the main sources of foodborne illnesses (Bhandare *et al.*, 2007). Specific sources that contribute microbial contamination to animal carcasses and to fresh meat during slaughter and dressing include the faeces, the hide, water, air, intestinal contents, lymph nodes, processing equipment, and humans (Sofos, 2005), and can be transferred to the carcass during skin removal and evisceration (Hansson *et al.*, 2000; Reid *et al.*, 2002).

The types of microorganisms and extent of contamination present on the final product are influenced by sanitation procedures, hygienic practices, application of food safety interventions, type and extent of product handling and processing, and the conditions of storage and distribution (Sofos, 2005).

There are four major pathogens that have frequently been associated with meat and meat products including *Salmonella* species, *Campylobacter* species, *Listeria monocytogenes*, and *Escherichia coli* O157:H7. These organisms have been linked to a number of cases of human illness (Mershal *et al.*, 2010). *Salmonella* is the most frequently reported cause of foodborne illness (Birhaneselassie and Williams, 2013). Foodborne salmonellosis often follows consumption of contaminated animal products, which usually results from infected animals used in food production or from contamination of the carcasses or edible organs (Alemayehu *et al.*, 2002). *Salmonella* infection in meat animals arises from intensive rearing practices and the use of contaminated feeds (Ejeta *et al.*, 2004). Cross-contamination of carcasses with *Salmonella* can also occur during slaughtering operations (Baird-Parker, 1990). Stress associated with transport of animals to abattoir augments shedding of *Salmonella* by carrier animals and this may contribute to the spread of the organism to other animals in the slaughter plant (Isaacson *et al.*, 1999).

Slaughtering procedures potentially involve many risks of both direct and cross contamination of carcasses and meat surfaces. During slaughter, faecal contamination of edible organs with subsequent contamination of the carcass may occur. This can be carried through all slaughter procedures up to the processing of the raw products, which are important sources of *Salmonella* in the human food chain (Edwards *et al.*, 1997). Contamination of equipment, utensils and hands of workers can spread *Salmonella* to uncontaminated carcasses and parts, which can occur in subsequent handling, processing, transport, storage, distribution and preparation for consumption (Ejeta *et al.*, 2004).

Salmonellosis causes significant morbidity and mortality in both humans and animals and has a substantial global socioeconomic impact (Tassios *et al.*, 1997). There are 16 million annual cases of typhoid fever, 1.3 billion cases of gastroenteritis and 3 million deaths worldwide due to *Salmonella* (Bhunia, 2008). Mortality due to *Salmonella* infections is mainly a health problem in developing countries, but morbidity due to acute *Salmonella* infections also has important socio-economic impact in industrialised nations (Hansen- Wester and Hensel, 2001). *Salmonella* infections in the United States account for roughly 19,336 hospitalizations, 17,000 quality adjusted life years lost and \$3.3 billion in total medical expenditures and lost productivity each year (Batz *et al.*, 2011). For human salmonellosis in the Netherlands, the costs are estimated to be between 32 and 90 million Euro per year (van Pelt and Valkenburgh, 2001).

Several methods have been developed for the detection, identification and molecular characterization of *Salmonella* species (Sen *et al.*, 2007). Culture can take from 4 to 7 days in order to isolate and confirm the presence of *Salmonella* from the sample (Bennett *et al.*, 1998). Conventional culture methods used for the isolation of *Salmonella* include, non-selective pre-enrichment followed by selective enrichment and plating on selective and differential agars. Suspected colonies are then confirmed biochemically and serologically. More recently, a number of alternative methods for the detection of *Salmonella* in foods have been developed including, immune-assays, nucleic acid hybridization and polymerase chain reaction (PCR) techniques (Li *et al.*, 2000).The Polymerase Chain Reaction (PCR) has become a powerful tool in microbiological diagnostics during the last decade. PCR based methods combine simplicity with a potential for high specificity and sensitivity in detection of foodborne pathogens.

The increase in the resistance of *Salmonella* to commonly used antimicrobials has been also noted in both public health and veterinary sectors (Molla *et al.*, 1999; Molla *et al.*, 2003 and Asrat, 2008). The extensive use of the first line drugs has led to the development of multiple drug resistance at a level which could pose a serious problem in the near future (Getenet, 2008). Although, little study has so far been undertaken to isolate *Salmonella* from small ruminat meat in Somaliland (Awo *et al.*, 2010, and Abdurahman *et al.*, 2008) from central part of the country and export abattoirs, there was no report regarding the status of the *Salmonella* from Sheikh Abattoir.

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Salmonella infections are very common and an important public health problem in many parts of the world. Studies in different countries indicated that Salmonellae are wide spread in small ruminants (Nabbut and Al-Nakhli, 1982 and Chandra *et al.*, 2007). Research to date, as well as unpublished reports from different health institutions in Sub-Saharan countries have indicated that salmonellosis is a common problem and also showed the presence of a number of serogroups/ serotypes in humans, animals, animal food products and other foods (Nyeleti *et al.*, 2000; Muleta and Ashenafi, 2001; Molla *et al.*, 2003; Tibaijuka *et al.*, 2003; Woldemariam *et al.*, 2005, Asrat, 2008 and Akafete and Haileleul, 2011).

The antimicrobial resistance of Salmonella is an increasing problem and has become a public health issue worldwide (Kaye et al., 2004). Antibiotics with the greatest percentage of resistant isolates include Amoxicillin, Clavulanic acid, Ampicillin, Ceftoifur, Cefoxitin, Chloremphenicol, Streptomycin, Sulfonamides, and Tetracyclines; however, the percentage of isolates resistant to these drugs has increased since 1997. Contamination of food with antibiotic-resistant bacteria can be a major threat to public health, causing community outbreaks of infectious diseases. Moreover the evidences on hazard of therapeutic failure due to the increasing incidence of antimicrobial resistance among Salmonella species are increasing now a day (Arslan et al., 2010). The general purpose of this study was to estimate the prevalence of Salmonella from sheep and goat meat slaughtered at Sheikh Abattoir. Specifically, it was aimed at; determining the prevalence and distribution pattern of salmonella spp between sheep and goat, isolating and identifying Salmonella from sheep and goat meat slaughtered at Sheikh Abattoir as well as delineating the antimicrobial sensitivity of the isolated pathogen.

2. METHODS AND MATERIALS

2.1 Study site:

This study was conducted between January 2018 and July 2018 in Sheik districts Slaughterhouse, Somaliland. Sheikh is in the centre of Somaliland and part of Sahil region. Physically the district lies between Latitudes 9° 56' 0" North & Longitudes 45° 11' 0" East. Sheikh District covers an area of 1,600 km2. Sheikh is located in the mountainous Golis Range between Berbera and Burao. It is 1,500 meters above sea level and has an average rainfall estimated to be 523 mm annually. It is suitable for livestock grazing and agricultural farming. Sheikh is an ideal place for tourism, beautiful weather and historical places. The district is bordered by Burao 65 KMs to the South, Odwayne 130 to the South West, Hargeisa 225.7 km to the Northwest, Berbera 75 km to the North, Sanaag 190 km to the East. The overall district population is estimated to be 42,560 persons of whom 8,600 people live in Sheikh City. Twenty-seven (27) villages come under its jurisdiction.



Figure 1: Map of the study area

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2.2 Study design:

A Case-study involving microbiological analysis and questionnaire survey was conducted from January 2018 up to July 2018 in Sheik districts Slaughterhouse, Somaliland. The format of the questionnaire survey is presented in (Annex).

2.3. Study population:

The study populations were all sheep and goats slaughtered from September 2018 to December 2018 at Sheikh Abattoir and meat handlers (abattoir workers, butchers and consumers).

2.4. Sample size determination:

For questionnaire survey the sample size was determined purposively based on the willingness of the interviewees, ease for follow up and the supply chain of small ruminants' meat from abattoir to consumers. Accordingly, 40 participants consisting of 20 abattoir workers and 20 butchers were included in the study.

For isolation and identification of Salmonella, the sample size will calculated according to Thrusfield, (2005), using a 95% CI, 5% precision and with an expected prevalence of 10%.

$$\mathbf{n} = \frac{\mathbf{t}2 * \mathbf{p} * (1 - \mathbf{p})}{\mathbf{d}2}$$

Where

n = required sample sizes

t = confidence level at 95% (standard value of 1.96)

P = estimated prevalence of carcass with Salmonellosis (10%)

d = margin of error at 5% (standard value of 0.05)

Therefore, (1.96)2*0.1(1-0.1)/(0.05)2 = 138.2976 which were approximated 139 carcasses.

To increases the precision of the estimate, the sample size was inflated and a total of 249 carcasses were considered.

2.5 Sampling technique and sample collection:

Carcasses samples were sampled by systematic random sampling technique. Swabs were taken according to the method described in ISO-17604 (2003). The thorax (lateral), front leg (shoulder), and hind limb (thigh), were the sampling sites. The sampling area was delineated by using a (10 x 10 cm) aluminum foil templates.

A sterile cotton tipped swab (2X3 cm) fitted with shaft, was first soaked in an approximately 10 ml of buffered peptone water (BPW) and rubbed over the delineated area horizontally and then vertically several times. Up on completion of the rubbing process, the swab was placed into the buffered peptone water used to wet the swab, breaking off the wooden shaft pressing against the inside of the universal bottle and disposed leaving the cotton swab in the universal bottle. Other swabs of the same types will be used on the other marked areas and placed into the same container. A second dry sterile cotton swab of the same type was used as before over the entire sampled area as above and this swab was placed into the same container. Finally, by using ice boxes with ice packs the samples were transported to IGAD Sheikh Technical Veterinary School.

2.6 Isolation and identification:

Salmonella was isolated and identified according to the technique recommended by the international organization for standardization (ISO-6579, 2002) as shown in (Annex.). The bacteriological media was prepared according to manufacturer's recommendations.

Vol. 6, Issue 4, pp: (1086-1103), Month: October - December 2018, Available at: www.researchpublish.com

2.6.1 Pre-enrichment and selective enrichment:

The swab samples were pre-enriched in appropriate amount of buffered peptone water in (1: 9) ratio and incubated at 37°C for 24 hrs. Rappaport- Vassiliadis medium (RV) broth and Müller Kauffman Tetrathionate with novobiocin (MKTTn) broth were used for selective enrichment of the samples. About 0.1 ml of the pre-enriched sample was transferred into a tube containing 10 ml of Rappaport- Vassiliadis medium (RV broth) and incubated at 42 °C for 24 hours. Another 1ml of the pre-enriched broth was transferred into a tube containing 10 ml of Rappaport- Vassiliadis medium (RV broth) and incubated at 42 °C for 24 hours. Another 1ml of the pre-enriched broth was transferred into a tube containing 10ml of MKTTn broth and incubated at 37°C for 24 hours.

2.6.2 Plating out and identification:

Xylose lysine desoxycholate (XLD) agar and brilliant green agar (BGA) plates were used for plating out and identification. A loop full of inoculums from each RV and MKTTn broth cultures were plated onto XLD and BGA plates and incubated at 37 0C for 24 hours. After incubation, the plates were examined for the presence of typical and suspect colonies.

Typical colonies of Salmonella grown on XLD-agar have a black centre and a lightly transparent zone of reddish colour due to the colour change of the media (ISO 6579,2002) while H2S negative variants grown on XLD agar are pink with a darker pink center. Lactose-positive Salmonella grown on XLD agar are yellow with or without blackening. Five typical or suspected colonies were selected from the selective plating media, streaked onto the surface of pre-dried nutrient agar plates and incubated at 37oC for 24hrs. Biochemical tests were done according to (ISO-6579, 2002) by using different biochemical tests that included TSI agar, L-lysine decarboxylation medium, urease and Indole production tests (Annex).

2.7 Antimicrobial susceptibility tests:

The antimicrobial susceptibility testing of the isolates was performed by using the disc- diffusion method according to the recommendations of the Clinical Laboratory Standards Institute (CLSI, 2012). 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 hours 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 inoculums and swabbed uniformly over the surface of Muller Hinton agar plate (Oxiod, England). The plates were held at room temperature for 30 min to allow drying.

The susceptibilities of the isolates were tested for the following antibiotic discs: Ampicillin (AMP) 10 μ g, Amoxicillinclavulanic acid(AMC) (30 μ g), Gentamicin (CN) 10 μ g, Ciprofloxacin (CIP) 5 μ g, Chloranphenicol (C) 30 μ g, , Sulphonamides (S3) 300 μ g, Tetracycline (TE) 30 μ g, Ceftriaxone (CRO) 30 μ g, Streptomycine (S) 10 μ g and Nitrofurans (F) 50 μ g, were placed at least 15 mm apart and from the edge of the plates to prevent overlapping of the inhibition zones. The plates were 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).

2.8 Data management and analysis:

The data collected from the questionnaire and observational survey and the results of the laboratory investigations was entered into Microsoft Excel and prepared for analysis. Descriptive statistics was performed using SPSS version 20 statistical.

3. PRESENTATION OF RESULTS

3.1 Prevalence of Salmonella spp in sheep and goat samples:

A total of 200 carcass swab samples comprising sheep (n=100) and goat meat (n=100) were screened for the presence of Salmonella spp. Out of them, 46 samples (32 sheep and 14 goat meat samples) were found positive for Salmonella spp by culture method with an overall prevalence of 23%. Significantly higher infection rate was recorded in sheep samples 34% (34 of 100) compared to goat samples 12% (12 of 100) (p < 0.05) with the likelihood of Salmonellosis occurrence 2.8 times higher in sheep than goat samples, as shown in (Figure 2).

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Figure 2: Prevalence of Salmonella spp. in sheep meat and goat samples.

3.2 Antibiotic sensitivity test:

Antimicrobial resistance has been recognized by World Health Organization as a major emerging problem of public health. The emergence and spread of multi-drug resistance against *Salmonella* species have reinforced the need for epidemiological studies describing the prevalence and the pattern of resistance of this strain. Different antibiotic sensitivity pattern was shown in the present study by different isolates

The highest level of resistance was observed for tetracycline (100%), nitrofurans (100%), Streptomycine (82.6%) and Ampicillin (52.2%). All isolates were susceptible to ciprofloxacin (100%) followed by gentmycin (91.3%) and Amoxicillin-clavulanicacid (71.73%) as shown in (Table 1). Sample wise antibiogram study revealed that all the isolates from goat meat were 100% resistant to Streptomycine whereas 76.5% of sheep meat isolates were resistant to Erythromycin. In case of Chloranphenicol the order of resistance in sheep was (55.9%)) and goat (.33%) (Table9).

SNo	Type of antimicrobial	Pattern of antibiogram		
		Resistant	Intermediate	Susceptible
1	Ampicillin (AMP) 10 μg,	24(52.2%)	3(6.5%)	19(41.3%)
2	Amoxicillin-clavulanicacid (AMC) (30µg)	4(8.7%)	9(19.6%)	33(71.73%)
3	Gentamicin (CN) 10 µg	0(0%)	4(8.7%)	42(91.3%)
4	Ciprofloxacin (CIP) 5 µg	0(0%)	0(0%)	46(100%)
5	Chloranphenicol (C) 30 µg	20(43.5%)	12(26.1%)	14(30.4%)
6	Sulphonamides (S3) 300µg	19(41.3%)	2(4.3%)	25(54.3%)
7	Tetracycline (TE) 30 µg	46(100%)	0(0%)	0(0%)
8	Ceftriaxone (CRO) 30 µg	10(21.7)	11(23.9)	25(54.3%)
9	Streptomycine (S) 10 µg	38(82.6%)	5(10.9%)	3(6.5%)
10	Nitrofurans (F) 50 µg	46(100%)	0(0%)	0(0%)

Table 1: Antibiogram assay of Salmonella isolates

Vol. 6, Issue 4, pp: (1086-1103), Month: October - December 2018, Available at: www.researchpublish.com

S.No.	Name of antimicrobial agent	Pattern of antibiogram	Sheep(n=34)	Goat(n=12)
1	Ampicillin (AMP) 10 µg,	R	15(44.11%)	9(75%)
		Ι	3(8.82%)	0(0%)
		S	10(29.41%)	9(75%)
2	Amoxicillin-clavulanicacid	R	2(5.88%)	2(5.88%)
	(AMC) (30µg)	Ι	4(11.76%)	5(41.67%)
		S	22(64.71%)	11(91.67%)
3	Gentamicin (CN) 10 µg	R	0(0%)	0(0%)
		Ι	4(11.76%)	0(0%)
		S	32(94.11%)	10(83.33%)
4	Ciprofloxacin (CIP) 5 µg	R	0(0%)	0(0%)
		Ι	0(0%)	0(0%)
		S	34(100%)	12(100%)
5	Chloranphenicol (C) 30 µg	R	19(55.9%)	1(8.33%)
		Ι	5(14.7%)	7(58.3%)
		S	11(32.35%)	3(25%)
6	Sulphonamides (S3) 300µg	R	10(29.41%)	9(75%)
		Ι	2(5.9%)	0 (00%)
		S	15(44.12%)	10(83.33%)
7	Tetracycline (TE) 30 µg	R	34(100%)	12(100%)
		Ι	0(0%)	0(0%)
		S	0(0%)	0(0%)
8	Ceftriaxone (CRO) 30 µg	R	7(20.59%)	3(25%)
		Ι	7(20.59%)	4(33.33%)
		S	16(47.05%)	9(75%)
9	Streptomycine (S) 10 µg	R	26(76.5)	12(100%)
		Ι	1(2.94%)	4(33.33%)
		S	0(0%)	3(25%)
10	Nitrofurans (F) 50 µg	R	34(100%)	12(100%)
		Ι	0(0%)	0(0%)
		S	0(0%)	0(0%)

Table 2: Sample wise antibiogram of Salmonella isolates

3.3 Questionnaire and observational survey:

3.3.1. Abattoir workers

Table 3 shows the knowledge, attitudes and practices of abattoir workers in relation to important parameters that potentially can influence the quality and safety of sheep and goat meat. All workers use unhygienic equipments and keep equipments in unhygienic places.

Factors	Values	Frequency	Percentage %
Educational status Illiterate		5	25
	Grade 1-4	13	65
	Grade 4-8	2	10
	Grade 8-12 and beyond	0	0
Placement in the abattoir Slaughtering, skinning evisceration splitting the carcass		18	90
	Management	2	10
Job related training	Yes	4	20
	No	16	80
Job related medical test	Yes	0	0
	No	20	100

Table 3: The knowledge, attitudes and practices of abattoir workers

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Know contamination as risk	Yes	7	35
	No	13	65
Unhygienic equipments	Yes	0	0
	No	20	100
Protective clothes	Yes	1	
	No	19	
Hand washing	Before work	5	25
	After the end work	13	65
	Before and after work	2	10

Whilst eight of the respondents responded that unclean hand and equipments as major causes of carcass contamination, six considered falling on the ground as a major source of contamination. Washing the hands before and after work is practiced by only two of the interviewees and nineteen did not regularly put on clean protective clothing at work (Table 3). None of them responded that the faeces, skin and dirty water could possibly cause carcass contamination.

Direct observations revealed the absence of hot water, sterilizer and carcass retention room in the abattoir. During slaughtering equipment are placed on unclean surfaces. Knives were placed on the floor, in their (workers) mouth, on the skin of killed and in the viscera of a slaughtered and hanged animals. The protective clothes were unclean, blood tinged and frequently in contact with carcasses.

3.3.2 Butchers:

Table 4 shows the knowledge, attitudes and practices of butchers in relation to important parameters that potentially can influence the quality and safety of sheep and goat meat. Among the twenty butchers, five acquired meat selling skills from observations and fifteen of them from informal training. Nineteen of the butchers did not use protective clothes and seventeen wash their hands with only water after work. All reported that they use a single knife for cutting meat and edible offal. Sixteen had worn jewelries and fourteen handled money while selling meat. All butchers cleaned their equipment every day at end of the selling process by using water and clothes but one reported that uses soap in addition to water and clothes.

Factors	Values	Frequency	Percentage %
Educational status	Illiterate	3	15
	Grade 1-4	11	55
	Grade 4-8	5	25
	Grade 8-12 and beyond	1	5
Received job related training	Yes	5	25
	No	15	75
Apron (protective clothes)	Used	1	5
	Not used	19	95
Jewellery materials	Worn	16	80
	Not worn	4	20
Handling money	Butcher with bare hand	20	100
	Cashier	0	0
Cutting table	Single	20	100
	Separate for different organs and meat		
	types		
Hand washing	Before work		
	After work	18	90
	During work		
	Not washed	2	10
Manner of hand washing	Rinsing with water only	17	85
	Using detergents and water	1	5
	Not wash	2	10
Use disinfectants	Yes	1	5
	No	19	95

Table 4: The knowledge, attitudes and practices of Butchers

Vol. 6, Issue 4, pp: (1086-1103), Month: October - December 2018, Available at: www.researchpublish.com

4. DISCUSSION

The prevalence of *Salmonellae* in sheep and goat carcasses was estimated at 34% and 12% respectively. The findings of the present study is higher to the previous reports from different authors (Akafete *et al.*, 2011; and Woldemariam *et al.*, 2005) who reported prevalence of *Salmonella* from goat carcass swab was 8.3% and 7.5% at, respectively. This difference could be due to differences in the hygienic and sanitary practices practiced in the abattoirs. Because, the current study concerns about the municipal abattoir, that has poor sanitation and hygienic standard in comparison to the export abattoirs.

In addition to this workers in the current abattoir were found to be with poor general and personal hygiene and lack of knowledge in hygienic processing of meat, due to lack of training regarding hygienic and sanitation of slaughtering and working environment generally and there was no disinfectants, hot water and separate room for final carcass and live animals in the abattoir. The overall high level of carcass contamination with *Salmonella* is of special public health significance for a country like Somaliland were most of the consumers does not have information about the risk of this contaminated meat, because they consider as it is safe to eat when slaughtered at abattoir therefore, consumers can also cross contaminate with other foods during processing.

Resistance to multiple antimicrobials (95.6%) which was observed in current study was higher than other studies conducted in some African countries. 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 the multidrug resistance of *Salmonella* isolated from food of animal sources, animals and humans, as well higher than reports from elsewhere in the world (Stevens *et al.*, 2006; Khaitsa *et al.*, 2007; Al-Bahry *et al.*, 2007; Elgroud *et al.*, 2009; and Fadlalla *et al.*, 2012), reported multidrug resistance of *Salmonella* isolates respectively as follow: 16%, 50% (from raw meats), (1.2%, 14.1% and 23.7%) *Salmonella* isolated from different type of samples, 51.7% and 37.82%. This difference could be because of that, antimicrobial-resistant *Salmonella* are increasing due to the use of antimicrobial agents in food animals at sub-therapeutic level or prophylactic doses 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; Molla *et al.*, 2006 and Zewdu and Cornelius, 2009).

Zewdu and Cornelius (2009) reported that the isolates of *Salmonella* from food items and workers from Addis Ababa were resistant to the commonly used antibiotics including streptomycin, ampicillin, and tetracycline. Furthermore, Zelalem *et al.*, (2011) also indicated resistance of *Salmonella* isolates to commonly used antimicrobials including ampicillin, streptomycin, and tetracycline, with resistance rate of 100%, 66.7%, and 33.3%, respectively. Similarly previous reports from South India (Suresh *et al.*, 2006), from Nigeria (Akinyemia *et al.*, 2005) and from Cameroon (Akoachere *et al.*, 2009) indicated a similar 100%, over 90% and 100% respectively resistance to ampicillin. The result of the current research also indicated resistance of *Salmonella* isolates to commonly used antimicrobials including tetracycline, nitrofurans, streptomycine, and ampicillin with resistance rate of 100%, 100%, 82.6%, and 52.2%) respectively. However, higher resistance rate than previous reports with the exception of ampicillin and resistance to further drugs as well as to Chloranphenicol and Sulphonamides with resistance rate of 43.5% and 41.3% respectively was observed in this result. This difference could be due to the increasing rate of inappropriate utilization of antibiotics which favors selection pressure that increased the advantage of maintaining resistance genes in bacteria (McGeer, 1998 and Mathew *et al.*, 2007).

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, in addition to public health problems, may lead to economic loss in the countries due to loss of exporting meat and animal products and cost of drug of choice to treat human and animals due to resistance development.

Ciprofloxacin showed a good antimicrobial activity against these *Salmonella* isolates. It was found that all of 44(100%) isolates were susceptible to ciprofloxacin. This result was comparable with previous reports by Molla *et al.*, (2006) from central part of Ethiopia among isolates of sheep and goat meat, Akinyemia *et al.*, (2005) from Nigeria, from human isolates and Zelalem *et al.*, (2011), isolates of *Salmonella* from dairy farms in Addis Ababa. The effectiveness of such drugs like ciprofloxacin could be because of that they are not widely used in countries like Somaliland and other African

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countries (Zelalem *et al.*, 2011). In addition to this, effectiveness of this drug could be because of this drug is not well distributed to all societies and not simply prescribed rather than it is used as drug of choice in antibiotic resistant person. In addition to this, ciprofloxacin is not commonly used to treat animals in Somaliland.

In the present study more than 60% of slaughter house workers and butchers had only a primary school education. Similarly more than 75% of slaughter house workers and butchers did not have job related training as regards to food hygiene but acquired their respective skills from observations. The results are in agreement with reports of Mekonnin *et al.* (2013) and Endale and Hailay (2013) who reported a primary school education and lack of job relating trainings in more than half of the slaughter house workers and butchers in Mekele city, Ethiopia. Therefore, these workers could cross contaminate and not handle meat hygienically due to lack of knowledge regarding hygiene, sanitation, risk of contamination and personal hygiene. However training of food handlers regarding the basic concepts and requirements of personal hygiene plays an integral part in ensuring safe products to the consumers (Adams and Moss, 1997) and food handlers should have the necessary knowledge and skills to enable them handle food hygienically (FAO, 1990).

The slaughtering process was unhygienic and unsanitary. There was no hot water, sterilizer and equipments rest on dirty surfaces. However, Akafete and Haileleul, (2011), reported that eviscerating knife significantly associated with carcass contamination and specific attention must be given to sterilization of knifes. Motsoela *et al.* (2002) also indicated that, it is salutary to note that knives must be immersed in water for two minutes at 82^oC to reduce the number of contaminating microorganisms. Contradictory to these facts, in current study site the same knife was used without sterilizing to slaughter different goats and sheep, for evisceration, cutting throat and skinning process. This could cause high carcass contamination with different foodborne pathogens unless it is solved.

Correspondingly, it was found that the equipment used for slaughtering process was rested on dirty surface during working, for instance they put their knife on ground, in their own mouth, on skin of other killed animal and in the anus of the hanged carcass and use it as it is, use the material they putted on the ground to collect water for washing carcass repeatedly, their protective clothes were full of blood and dirty and were in contact with carcass while they take the finalized carcass to the final loading. Therefore the risk of carcass contamination might be increasing until it reaches the consumers at different stage due to above listed predisposing factors such as in contact with dirt clothes wile loading, transportation, contaminated water in use of contaminated materials repeatedly and moving from one hook to another hook.

The hygienic practices at the butcheries are unhygienic. All butchers (100%) handle money with bare hands while processing meat and do not put appropriate protective clothes. In addition other study indicates that, Handling of foods with bare hands may also result in cross contamination, hence introduction of microbes on safe food. Because meat handlers are probable sources of contamination for microorganisms, it is important that all possible measures be taken to reduce or eliminate such contamination (Muinde and Kuria, 2005). In addition almost all butchers (90%) wash their hands after the selling process and use only water with no detergents and use single knife for edible offals and meat types and a single cutting board for all products without cleaning and sterilizing.

5. CONCLUSION

The overall prevalence of *Salmonella* was found 34% in sheep whereas 12% in goat meat in this study area with little variation in biochemical activities of the isolate. *Salmonella* needs special concern because of poor hygienic conditions prevailing in the areas of sampling which ultimately favour its spread. *Salmonella* spp. should be under supervision of public health and veterinary authorities to ensure the early detection and to implement preventive measures to control the spread of zoonoses. The present study results revealed that high prevalence of *Salmonella*, presence of poor personal hygiene and sanitation, resistance of *Salmonella* to most antimicrobials except ciprofloxacin, low level of public awareness about contamination of sheep and goat meat with *Salmonella* and the associated probable risk in the study area. Consequently, sheep and goat meat provided to the consumers in the city was found to be poor quality and not safe for human consumption calling for urgent intervention.

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Vol. 6, Issue 4, pp: (1086-1103), Month: October - December 2018, Available at: www.researchpublish.com

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