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Research Article

Assessment of the Contamination of Beef with Salmonella along Beef Supply Chain in Dukem Town, Ethiopia

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Abstract

Salmonella is a major cause of food borne disease in the world, with an increasing concern for the emergence and spread of antimicrobial-resistant strains. A cross-sectional study was conducted between November 2016 and April 2017 in Dukem town. The purpose of the study was to determine the prevalence and identify the antimicrobial susceptibility pattern of *Salmonella*. Following the accepted methodologies and procedures, a total of 286 samples, including feces, carcass swabs, and retail meat, were collected and tested for Salmonella. Systematic random sampling and purposive sampling techniques were used to generate the desired data. *Salmonella* was present in the beef supply chain on average at a rate of 6.3% (95% CI: 3.9-9.7). Based on sample source, the specific incidence of Salmonella was 0.9%, 2.9%, and 12.7% in retail meat, feces, and carcass swabs, respectively. There was statistically significant difference along the beef supply chain (X2 = 14.3027, P < 0.05). Among the isolates, 94.4% (n = 17) were resistant at least to one of the antimicrobials. Multi-drug resistance was observed in 27.8% (n = 5) of the isolates. The study found the occurrence of *Salmonella* along beef supply chain with higher prevalence at meat retail shop and the variability in the susceptibility pattern of *Salmonella* isolates against the tested antimicrobials. Identifying *Salmonella* serotypes circulating in the area and regular monitoring of the health status of workers and hygienic condition of the slaughterhouse and meat retail shop is recommended.

Keywords: Antimicrobial Resistance; Beef; Contamination; Dukem; Prevalence; *Salmonella*

Introduction

Food borne diseases(FBD) are diseases of infectious or toxic nature caused by the consumption of foods or water contaminated with bacteria and/or their toxins, parasites, viruses, or chemicals [1].

There are many and varied sources of organisms causing FBD. Most cases are caused by bacteria which arise from animal, human or environmental sources [2]. *Salmonella* species, *Campylobacter* species, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 linked to illness due to consumption of meat and meat products [3].

With estimates of 22 million cases and 200,000 fatalities from typhoid fever and 93.8 million episodes of gastroenteritis and 155,000 deaths from non-typhoidal *Salmonella*e (NTS), salmonellosis is one of the most common zoonotic illnesses in the world [4]. Currently known *Salmonella* serotypes number over 2,700. Many are known to cause illness in humans [5]. One of the most significant causes of foodborne illness, Nontyphoidal *Salmonella* (NTS) spp. produces diarrhea, bacteraemia and focal suppurative infections. *S.* enterica sub sp.enterica with *S.*Enteritidis and *S.*Typhimurium were responsible for most of the infections associated to humans and other mammals [6].

Characteristics of *Salmonella*

Salmonella is a genus of the family Enterobacteriaceae. It is a Gram-negative, non-spore-forming, rod-shaped and facultative anaerobic bacterium. *Salmonella* cells move by means of a peritrichous flagellum. They are 2-5 µm long by 0.5-1.5 µm wide and, depending on the serotype, the *Salmonella* genome ranges from 4460 to 4857 kb. The bacterium was first identified in a veterinary laboratory in the 19th century in the USA. *Salmonella* is a lactose fermenter (some sub-species) and a hydrogen sulfite producer and is oxidase-negative and catalase-positive. It hydrolyzes urea, utilizes citrate and decarboxylates lysine as its sole carbon source [7,8].

The genus is classified into two species: *Salmonella* enterica and *Salmonella* bongori. Biochemical and genomic analysis of *Salmonella* enterica has led to further classification into subspecies, including enterica, salamae, arizonae, diarizonae, houtenae and indica [8-10]. The clinically important *Salmonella* species are classified under *Salmonella* enterica, which is further classified into more than 2,579 serovars on the basis of their antigenicity [8,11].

Salmonella species are harboured in the intestinal tract of humans and farm animals. Reptiles and insects also act as *Salmonella* reservoirs. Moreover, eggs, poultry meat, pork, beef, dairy products, nuts, vegetables and water act as sources of *Salmonella*. The risk of infection is high in low- and middle-income countries or societies, with more than 100 infections per 100 000 people per year [8,12-14]. Some *Salmonella* serotypes are host-specific, while others can infect more than one type of warm-blooded animal [7]. The S. Typhi and *Salmonella* enterica serovar Gallinarum serovars are restricted to human and poultry hosts, respectively, whereas *Salmonella* enterica serotype Dublin (S. Dublin) and *Salmonella* enterica serovar Choleraesus are adapted to cattle and pigs, respectively, but can infect other warmblooded animals. However, other serovars, such as *Salmonella* enterica serovar Typhimurium (S. Typhimurium) and *Salmonella* enterica serovar Enteritidis (S. Enteritidis), are generalists and are able to infect any warm-blooded animal [7].

The bacterium can be transmitted through faecal-oral routes, where susceptible hosts may acquire *Salmonella* through contaminated foods and water and therefore transmissions can be controlled through foods and water [12]. Moreover, infection with *Salmonella* from food or water can also be prevented with vaccination. *Salmonella* vaccines include killed whole-cell, Vi, live oral Ty2la and Vi-rEPA. The use of vaccine may reduce infections, but availability, efficacy, safety and cost are some of the issues that hamper its use and effectiveness [14,15].

Of the NTS, *Salmonella* Typhimurium and *Salmonella* Enteritidis account for nearly 80% of all human isolates reported globally [16].

The main sources of meat contamination include; animal/carcasses source, on farm factors, transport factors, abattoir and butchers facilities, parasites and wild animals, meat van, abattoir and retail meat outlet workers [17]. The majority of transmission occurs through contaminated food, however there are also alternative routes such as drinking tainted water, coming into contact with sick animals, and nosocomial exposure [18].

Moreover, antimicrobial resistant *Salmonella* are becoming challenge. Antimicrobial resistant strains could develop as a result of using antimicrobial drugs in food animals at prophylactic or subtherapeutic dosages, significantly raising the danger to human health from consuming tainted meat products [19,20]. Antibiotic resistantNTS are associated with increased treatment failure and risk of invasive disease [21].

Food animals harbor a wide range of Salmonella serotypes and so act as source of contamination which is of paramount epidemiological importance in non-typhoid human salmonellosis. Stress related to animal transportation to the abattoir increases the amount of Salmonella excreted by carrier animals, which may help the pathogen spread to the corpse during the slaughter process [22].

The process of removing the gastrointestinal tract during slaughtering of food animals is regarded as one of the most important sources of carcass and organ contamination with *Salmonella* at abattoirs [23].

Salmonella contamination in beef can occur at several stages along food supply chain includes productions, processing, distribution, retailing and also preparing and handling by consumers [24].

Despite the presence of many studies on salmonellosis in different parts of Ethiopia [23,26-27] there is no citable information regarding to the status of Salmonella along beef supply chain in Dukem town where raw beef consumption is prediminantly common.

Therefore, this research was conducted in the aim of identifying the antibiotic susceptibility pattern of *Salmonella* isolates and estimating the prevalence of *Salmonella* along the beef supply chain and determining the antimicrobial susceptibility pattern of *Salmonella* isolates.

Material and Methods

Study area

Dukem town is located at 37 km Southeast of Addis Ababa along the main road to Adama. Geographically, the research area spans 35.96 km2 and is located between latitudes 8o45’25”N and

8o50'30"N and longitudes 38o51'55"E and 38o56'5"E. It is situated at a typical elevation of 2100 meters above sea level [28]. According to meteorological data from 1996 to 2003 at the Bishoftu station, the area's mean annual rainfall is 606.13 mm, and its mean maximum and mean minimum annual temperatures are 25.83 °C and 11.9 °C, respectively. Mid-October to January sees the lowest temperature while February to May sees the highest. Dukem Town has a population of 24,222 people, according to the 2007 Census of Population and Housing [29]. Population and study design to gather the required data along the beef supply chain in the study area, a cross-sectional study design was used. Study design and population.

A cross-sectional study design was employed to generate the desired data, along the beef supply chain in the study area.

The study populations were cattle slaughtered in Dukem Municipal abattoir. Cattle presented to abattoirs originated from Dukem town and its surroundings. The daily slaughtering capacity of the is 25-30 bovines in regular basis but this number increased to around 95 bovine per day in holidays. The average carcass weight of individual bovine is 212 kg.

Sample size determination

The sample size was determined using the formula described by Thrusfield [30] by assuming 5% precision, 95% level of confidence interval and 7.07%, 4.53% and 8.34% expected prevalence of *Salmonella* in cattle feces, [31] carcass at slaughterhouse and meat at market, [26] respectively.

$n = Z^2 p \exp (1\text{-}pexp)/d^2$

Where n = required sample size; pexp = expected prevalence and d = desired absolute precision of 0.05, Z = 1.96

Accordingly, the minimum sample sizes required from each points of beef supply chain were 101, 67 and 118 for feces, carcass and retail meat, respectively.

Sampling technique

Sample animals from abattoirs were selected by systematic random sampling technique. Identification number was given for each animal for selection during ante mortem examination and follow up during postmortem examination, depending on the number of animals slaughtered on each day [30].

Fecal samples were taken immediately from rectum after evisceration of the identified animals. The rump, flank, brisket and neck were sample site for beef carcasses swabbing in the abattoir. A sterile cotton tipped swab (2 × 3 cm) fitted with wooden shaft was first soaked in 10 ml of sterile buffered peptone water (BPW) (OXOID, England) [32]. At each of these four sample sites, samples were obtained by rubbing the applicator stick ten times in a horizontal manner and ten times in a vertical direction over a 100 cm2 area. Carcass swab samples were collected once at the final slaughtering process before it was prepared for loading and inserted into the universal bottles containing10 ml BPW after cutting off the part the stick which was in contact with the hand, by binding out on the mouth of bottle [23]. Meat cut sample (30g) from meat shops also collected and put in sterile cups.

Isolation and Identification

According to the International Organization for Standardization's established methods, *Salmonella* was isolated and identified [33]. *Salmonella* must be detected using four sequential steps, including pre-enrichment in non-selective liquid media, enrichment in selective media, plate out and identification, and biochemical confirmation of suspicious colonies, according to this ISO standard. Test for Antimicrobial Sensitivity To ascertain the antibiotic-resistant profiles of each isolate, phenotypic antimicrobial susceptibility testing on Mueller-Hinton agar (Oxoid) utilizing the agar disc diffusion method [34] was carried out.

Ampicillin (25 mg), cefoxitin (30 mg), nalidixic acid (30 mg), nitrofurantoin (50 mg), sufisoxazole (100 mg), tetracycline (30 mg), Kanamycin(30mg), Streptomycin (10 mg) and Trimethoprim (25mg) were used.

Four to five well isolated colonies grown on nutrient agar were transferred on to tubes containing 5ml of citrate broth. The broth culture inoculated at 37^0C for 4hrs until it achieves or exceeds the 0.5 McFarland turbidity standard. For those tubes which exceeded the turbidity standard, adjustment was made by adding the sterile saline solution to obtain turbidity usually comparable to the standard.

Following incubation, the diameters of the inhibition zone were measured in millimeters and interpreted in accordance with CLSI guidelines [34]. The zone of inhibition was measured and report-

ed as susceptible (S) intermediate (I) or resistant (R) in reference to performance standards for antimicrobial susceptibility testing of *Salmonella.* If the isolates were resistant to two or more of the antimicrobial agents tested, they were judged to have developed multiple drug resistance (MDR).

Data management and analysis

Data was coded, entered, and managed by Microsoft Office Excel spread sheet (2007) and analyzed by using STATA release 11 computer software. Descriptive statistics (frequency and percentage) was used to summarize the result. Chi square test with 95% confidence interval was used to assess the association of the sample sources with the *Salmonella* positivity and the difference in the proportion of *Salmonella* in the chain of beef supplier. P-value less than 0.05 was considered statistically significant [35].

Results

286 samples in all, including of 118 retail meats, 67 carcass swab and 101 fecal samples were collected and analyzed. Among these samples examined, 6.3% (n = 18) were positive for *Salmonella*. The highest sample prevalence (12.71%) was found on meat at retailer which contributed 83.3% (15 of 18) of total isolates while the lowest prevalence 0.9% (1 of 101) was found on fecal sample contributed 5.5% (1 of 18) of total isolates (Table 1).

Sample types	Number of samples	Positive (%)	95%Conf. Interval
Feces	101	1 (0.99%)	0.1-6.9
Carcass swab	67	2 (2.98%)	0.8-11.5
Meat	118	15 (12.71%)	7.8-20.1
Total	286	18 (6.29%)	

Table 1: The occurrence of *Salmonella* from different sample types.

Source: Self (2017, Laboratory findings).

Prevalence of *Salmonella*

The result shows statistically significant variation in the prevalence of *Salmonella* along the supply chain for beef ($X^2 = 14.3027$, p-value = 0.001).

Antimicrobial susceptibility of *Salmonella* isolates

All the 18 isolates were tested against nine commonly used antibiotics and Kanamycin was sensitive to every isolate (100%) as opposed to 94.4%, 88.9%, and 83.3% of the isolates were found to be sensitive to Sufisoxazole, Tetracycline and Nalidixic acid, respectively (Table 2). tested. The *Salmonella* species isolated along Dukem's beef supply chain exhibited resistance against 6 out of the 9 antibiotics used in this study. 94.4% of the isolates (n = 17) were at least partially resistant to one of the antibiotics. In this regard, 72.2%, 33.3%, 22.2%, and 22.2% of the isolates, respectively, were resistant to ampicillin, nitrofurantoin, cefoxitin, and trimethoprim. (Table 2).

Type of Antimicrobials	Number of isolates		
	Resistant (%)	Intermediate (%)	Susceptible (%)
Trimethoprim (TR)	4 (22.2%)	2 (11.1%)	12 (66.7%)
Nalidixic acid (NA)	-	3 (16.7%)	15 (83.3%)
Kanamycin (K)	-	-	18 (100%)
Cefoxitin (FOX)	4 (22.2%)	4 (22.2%)	10 (55.6%)
Streptomycin (S)	3 (16.6%)	7 (39%)	8 (44.4%)
Nitrofurantoin (F)	6 (33.3%)	5 (27.7%)	7 (39%)
Sufisoxazole (RL)	-	1 (5.6%)	17 (94.4%)
Ampicillin (AMP)	13 (72.2%)	4 (22.2%)	1 (5.6%)
Tetracycline (TE)	2 (11.1%)	-	16 a(88.9%)

Table 2: Antibiotic Susceptibility Profiles of *Salmonella* isolate.

Source: Self (2017, Laboratory findings).

Most isolates in the group (83.3%, n = 15) originated from retail meat and exhibited different antimicrobial and multi-drug resistant patterns. The isolate that was recovered from feces was found to be resistant to Ampicillin, Nitrofurantoin and Cefoxitin. All isolates recovered from carcass swab were able to resist ampicillin but 50% of these isolates exhibited anti-to Nitrofurantoin, Trimethoprim and Cefoxitin (Table 3).

From the 18 isolates, 9(50%) isolates had two or more drug resistance, and from those 5 (27.8%) isolates were showed multi-drug resistant (resistance for three and more antibiotics) (Table 3).

Type of Antimicrobials	Number of resistance *Salmonella* isolates			Total
	Retail meat (%) (n = 15)	Carcass swab (%) (n = 2)	Feces (%) (n = 1)	
Ampicillin	10 (66.7)	2 (100)	1 (100)	13 (72.2%)
Nitrofurantoin	4 (26.6)	1 (50)	1 (100)	6 (33.3%)
Trimethoprim	3 (20)	1 (50)	-	4 (22.2%)
Cefoxitin	2 (13.3)	1 (50)	1 (100)	4 (22.2%)
Streptomycin	3 (20)	-	-	3 (16.6%)
Tetracycline	2 (13.3)	-	-	2 (11.1%)
Sufisoxazole	-	-	-	-
Nalidixic acid	-	-	-	-
Kanamycin	-	-	-	-

Table 3: Resistance pattern of *Salmonella* isolates according to the sample sources.

Source: Self (2017, Laboratory findings).

The common multiple resistance pattern was to the combination of Nitrofurantoin, Ampicillin and Trimethoprim, seen in 2 (11.1%) of the resistant isolates (Table 4).

Number of antimicrobial resistances	Antimicrobial resistance patterns (number of isolates)	Number of isolates (%)
One	AMP (6), TE (1), S (1)	8 (44.4)
Two	FOX+AMP (2) F+TE (1) TR+S (1)	4 (22.2)
Three	TR+F+AMP (2) S+F+AMP (1) FOX+F+AMP (1)	4 (22.2)
Four	TR+F+AMP+FOX (1)	1 (5.6)

Table 4: Multiple antimicrobial resistance profile of *Salmonella* isolates.

Source: Self (2017, Laboratory findings).

Key: AMP: Ampicillin; S: Streptomycin; FOX: Cefoxitin; F: Nitrofurantoin; TE: Tetracycline; TR: Trimethoprim

Discussion

The prevalence of *Salmonella* was examined in the current study in raw retail meat samples was 12%, this was in close agreement with the report of, [23] who reported 12% from retail raw meat samples in Addis Ababa, Ethiopia. But the current finding lower in comparison with the studies conducted in Senegal, 87% [36] and in Iran, 47% [37] This difference might be the sample type and sample procedures and the detection methods employed for different studies. As Padungtod and Kaneene [38] described earlier, the prevalence may also differ from study to study, from one nation to another, or from one region to another within a single nation.

In the current research prevalence of *Salmonella* in feces and carcass was found to be 0.9% and 2.9% respectively.

Of the feces samples analysed 0.9% was positive for Salmonella and agreed with [34] who reported that the prevalence of 1.9% in apparently healthy slaughtered cattle. The current study revealed prevalence of Salmonella, 2.9% in carcass swab samples, this result was lower than 8% reported by [36] in Wolaita Sodo municipal abattoir. The difference in the prevalence reported could result from differences in study sites (abattoirs) and animal.

In the current research, all *Salmonella* isolates tested were found to be resistant to a minimum of antimicrobial agent. This observed resistance profile was higher than what other studies reported in Ethiopia (23.5%), [39] in Senegal (17%) [27] and 83% reported in Thailand [40] Resistance was noted to four, five, six, seven, and more antibiotics at varying proportions [41] and 52% of the *Salmonella* isolated at the abattoir from beef had at least three antibiotic resistances [42].

Even though it needs a better understanding of antibiotics use in Ethiopia, this resistance variation might be due to indiscriminate use of antimicrobials in animal production without prescription in the animal health sector, which might favor selection pressure that increased the advantage of maintaining resistance genes in bacteria [43]. The emergence of antimicrobial-resistance to *Salmonella* is associated with supplement of antibiotics to animal feed and for their treatments. Resistant bacteria can be transmitted to humans through foods, particularly those of animal origin [44].

In this study, majority (55.6%) of the identified isolates had two or more drug resistance antimicrobials, particularly to Ampicillin (72.2%), Trimethoprim (22.2%), Nitrofurantoin (33.3%). *Salmonella* resistance in this finding was higher than the earlier research done in Ethiopia [30,45] and other countries [28,32].

The development of multiple drug resistance by the isolates is serious concerns that there may critical challenge for treatments of the salmonellosis. There were a number of studies where multiple drug resistance of *Salmonella* against antibiotics reported. The remarkable rise in the occurrence of antimicrobial resistance in *Salmonella* for the mentioned antibiotics was probably an indication of their frequent usage both in livestock and in public health sectors in Ethiopia. The systematic review and meta-analysis in Ethiopia [46] indicated the increase in the proportion of drug-resistant *Salmonella* isolates that could be due to the irrational use of antimicrobials and inappropriateness of the prescription and dispensing methods in both the public veterinary and private health setups of the country.

Due to the relatively limited access and high price to get the newly developed cephalosporin and quinolone drugs, the reports of prevalence of antimicrobial-resistant *Salmonella* to relatively low-priced and regularly available antibiotics are alarming for a low-income society living in most developing countries, like Ethiopia. However, it is important to note that these antibiotics are commonly used in veterinary medicine, and infections with these resistant *Salmonella* isolates could lower the efficiency of antibiotic treatment. The finding of this study shows slightly lower resistance than the study reported in Nigeria (93.1%) [47].

Resistance to multiple antimicrobials (55.6%) which was observed in current study was higher than other studies conducted in Ethiopia. For instance, [48] reported 52%, 23.5%, and 44.8% 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 [36], 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 [49,39].

The isolates of *Salmonella* from food items and workers from Addis Ababa were resistant to the commonly used antibiotics including streptomycin, ampicillin, and tetracycline [39]. Furthermore, [19] also indicated resistance of *Salmonella* isolates to commonly used antimicrobials including ampicillin, streptomycin, Nitrofurantoine, Kanamycine and tetracycline, with resistance rate of 100%, 66.7%, 58.3% and 33.3%, respectively.

Conclusions and Recommendations

The present study showed that *Salmonella* outbreaks in the beef supply chain and its public health importance unless the necessary intervention is in place. More specifically, it revealed high contamination of retail meat with *Salmonella*, the variation in *Salmonella* isolates' susceptibility profile against the examined antimicrobials. Similarly, multiple drug resistant *Salmonella* isolates were found to occur in the study area.

Further study should be conducted to identify the source of contaminations, identify *Salmonella* serotypes, circulating in the area, molecular characterization of the resistant *Salmonella* isolates to better understand the underlying the resistant genes and elucidate mechanisms of resistance development should be undertaken.

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