

Research Article

Occurrence of Salmonella and its antimicrobial sensitivity test in Abattoir and Dairy farms in Adama town, Oromia, Ethiopia

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Abstract

A cross-sectional study was conducted on dairy farms and cattle slaughtered in Adama municipal abattoir in Adama town from October 2013-April 2014 to isolate, identify and assess antimicrobial sensitivity profile of *Salmonella* in abattoir and dairy farms. A total of 182 samples consisting of feces from farm (n= 36), bucket milk (n=36), tank milk (n= 6), tank swab (n= 4), bucket swab (n= 5), hand swab from milker (n= 6), feces from abattoir (n= 24), mesenteric lymph node swab (n= 24), carcass swab (n= 27), pooled knife swab (n = 5), pooled hanging material swab (n= 5) and hand swab from butcher (n= 5) were collected separately. The samples were examined for the presence of *Salmonella* following the standard techniques and recommended by the International Organization for Standardization (ISO) via culturing on bacteriological media and testing using a series of biochemical tests. Accordingly, out of a total of 182 samples, 11(6.04%) were *Salmonella* positive in that 1(3.7%) in carcass swab, 3(12.5%) in mesenteric lymph node swab, 1(20%) in pooled knife swab, 3(12.5%) in feces from abattoir and 3(8.6%) in feces from farm. No statistical significant association ($p>0.05$) could be obtained between bacteriological status of sample sources and sample types. Antibiotic susceptibility testing was undertaken using disc-diffusion test. All of the isolates were tested for susceptibility to ten antimicrobials. Out of 11 isolates that were tested for antimicrobials 10(91%) of them were resistant to at least one or more antimicrobial agents. An isolates was considered as multiple drug resistant if it is resistant for 3 and more drugs. Multiple antimicrobial resistances were demonstrated for 6(54.5%) of isolates. Most frequent resistance was encountered for Streptomycin (72.7%), Cefoxitin (63.6%) and followed by Ampicillin (54.5%). Results of this study showed that *Salmonella* were spread in abattoir equipment, cattle feces and mesenteric lymph node. The study also indicated the need for further studies to determine risk factors associated with the epidemiology and antimicrobial resistance of *Salmonella*. Furthermore, appropriate measures should be taken to reduce its infection and contamination in dairy farm and thereby minimize the potential food-borne *Salmonella* infection in man.

INTRODUCTION

Food safety has been a concern of mankind since the dawn of history. Despite advance in food science and technology, food borne diseases are among the most widely spread global public health problems of recent times and their implication for health and economy is increasingly recognized. The world Declaration on Nutrition adopted by FAO/WHO International Conference on nutrition emphasizes that hundreds of millions of people suffer from communicable and non-communicable diseases caused by contaminated food and water [1-3]. Food borne diseases occur commonly in developing countries particularly in Africa because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest in safer equipment and lack of education for food-handlers. Wide spectrum of pathogen play major role in causing food borne disease. Most of them are zoonotic and have reservoirs in health food animals from which they spread to variety of foods. Therefore, foods of animal origin are considered as major vehicles of food borne infections [4]. In Ethiopia, the widespread habit of raw beef consumption is a

potential cause for food-borne illnesses besides, the common factors such as overcrowding, poverty, inadequate sanitary conditions, and poor general hygiene. Raw meat is available in open air local retail shops without appropriate temperature control and this is purchased by households and also minced meat (Kitfo) is served at restaurants as raw, slightly-cooked or well-cooked. Among the food-borne pathogens the genus *Salmonella* is one of the most common causes of food-borne infections worldwide. Salmonellosis is one of the most common and widely distributed food-borne diseases associated with food of animal origin and are caused by the bacteria *Salmonella*. *Salmonella* is a genus of Gram-negative rod-shaped bacteria of the family Enterobacteriaceae [5]. In many registers non-typhoid *Salmonella* species are documented as one of the leading causes of bacterial diseases. Food borne *Salmonella* typically causes acute gastroenteritis and may cause a more septicemia disease usually in very young; the elderly and immune-compromised subjects [2].

Salmonella species occur widely in natural environment and in different sectors of the global food chain. The ability of these

microorganisms to survive under adverse conditions and to grow in the presence of low level of nutrients and at sub optimal temperatures and pH values presents a formidable challenge to the agriculture and food processing industries in marketing safe products. The continued prominence of raw meats, eggs, dairy products, vegetables sprouts, fresh fruits, and fruit juices as the principal vehicle of human food borne salmonellosis arises from major difficulties to coordinate sectorial control efforts within each industry. The problem of Salmonellosis is further compounded by the massive and unrestricted movement of food in international trade, the national disparities in the hygienic agricultural and aquaculture production of foods and the non-uniform government and industry food safety controls during the processing, distribution and marketing of fresh and processed food products [6]. Food animals harbor a wide range of *Salmonella* serotypes and so act as a source of contamination, which is of paramount epidemiological importance in non-typhoid human salmonellosis [2]. Cattle can be chronically infected and serve as carriers within the herd without exhibiting clinical signs. *Salmonella* shed in the feces of livestock such as cows and goats and can contaminate milk during the milking process [7]. Humans and other animals can become infected from consumption of contaminated drinking water, raw dairy and milk products, and undercooked meat products [8].

There is no practical means of detecting slaughter animals with a subclinical infection. Each infected animal is a potential source for the spread of *Salmonella* in the abattoir [9]. 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. 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 [10].

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 [11]. Antimicrobial compounds have been used to treat bacterial infections since the middle of the twentieth century. These compounds were highly successful in treating various diseases and were widely used in both human and veterinary medicine. However, resistance to these compounds was detected in target pathogens only a few years after initiation of therapeutic use in humans [12]. Generally, mechanisms of antimicrobial resistance fall into three categories: (1) inactivation of the antimicrobial, (2) efflux or changes in permeability or transport of the antimicrobial, or (3) modification or replacement of the antimicrobial target [13-16]. Control of antibiotic resistant *Salmonella* is most efficiently through the reduction of consumption antibiotic. Control of animal feed, husbandry, hygiene in abattoir routinely, sanitation at all stages and food services are ways to minimize the need for antibiotic treatment [17].

Despite a major zoonotic, food-borne and pathogen with high drug resistant along with diversity in its strains and host ranges, there is limited study on *Salmonella* in Ethiopia. Therefore, the objectives of this study were to isolate and identify *Salmonella* in abattoir and dairy farms and to evaluate the antibiotic sensitivity profile of *Salmonella* isolates.

MATERIALS AND METHODS

Study area

Adama is a city in central Ethiopia and the previous capital city of the Oromia Regional state. Adama forms a Special Zone of Oromia and is surrounded by East Shewa Zone. It is located at 8.55°N 39.27°E at an elevation of 1712 meters, 99 km southeast of Addis Ababa. The city sits between the base of an escarpment to the west, and the Great Rift Valley to the east. Based on the 2007 Census conducted by the Central Statistical Agency of Ethiopia (CSA), it has a total population of 220,212, an increase of 72.25% over the population recorded in the 1994 census, of which 108,872 are men and 111,340 women. With an area of 29.86 square kilometers, Adama has a population density of 7,374.82; all are urban inhabitants. A total of 60,174 households were counted in this city, which results in an average of 3.66 persons to a household, and 59,431 housing units.

Study population

In Adama town there are many individual farmers and milk producers dairy farms. The five dairy farms from which the study was investigated have 6-35 lactating Holstein-Friesian cross-bred cows. The raw milk produced by individual farmer from local lactating cows is consumed by many farm families in their home whereas the dairy farm owners brought the milk to local consumers, restaurants and cafeterias at Adama town. The Adama municipal abattoir is one of the modern abattoirs constructed in Adama town. In the abattoir 15 to 240 animals originating from different area with different management system slaughtered per day (personal communication). The meat from this abattoir distributed to Adama city and Adama Science and Technology University.

Study design and sample collection

A cross sectional study design was conducted in this research. The sample was collected from February 2014 to April 2014. A total of 182 samples consisting of feces from farm (n= 36), bucket milk (n=36), tank milk (n=6), tank swab (n= 4), bucket swab (n= 5), hand swab from farm (n = 6), hanging material swab (n =5), pooled knife swab (n = 5), carcass swab (n= 27), lymph node swab (n = 24), hand swab from abattoir (n = 5), feces from abattoir (24) were collected from Adama municipal abattoir and dairy farms in and around Adama town. Fecal sample was collected from the rectum of animals in both farm and abattoir. Pooled bucket milk taken directly from udder four teats to test tube containing pre-enrichment media (buffered peptone water) in a ratio of 1:9. Tank milk was sampled after the milking process completed and milk from all cow collected in one container. Tank swab and bucket swab taken before milking using sterile swab applicator and put in the pre-enrichment media. Hand swab was taken from milkers and slaughter man after milking and slaughter finished but before washing. Pooled hanging material swab

was sampled from a material through which carcass passed until transported. Carcass swab taken from frequently contaminated while dressing (neck, thoracic, and thigh muscle). Lymph node swab collected from animal which fecal sample was taken after incised by sterile scalpel blade. The samples were transported to the laboratory after being collected in a portable container with ice packs (at 4°C) and microbiological analysis was carried out immediately.

Isolation and identification of *Salmonella* organisms

The isolation of *Salmonella* was performed according to the standard operating procedure set by the Global *Salmonella* Surveillance and laboratory support project of the World Health Organization (WHO) and the National Health Services for Wales (NHS), in which both procedures use ISO-6579 ISO, (2002) Standard for the isolation of *Salmonella*. Pure cultures obtained from nutrient agar were tested biochemically according to ISO 6579 (2002) (ISO, 2002). Samples were dispersed into suitable non-selective medium (buffered peptone water). Zero point five and One militer of the pre-enrichment culture was transferred into selective enrichment broth (10 mL Rappaport-Vassiliadis soy peptone (RVS) and 10ml of Selenite F broth) respectively. In the Selenite F broth the sample was incubated at 37°C for 24 - 48 hours. Subsequently, the enriched sample was streaked onto each of the *salmonella* shigella agar (SS) and Xylose Lysine Deoxycholate agar (XLD) and incubated at 37°C for 24 h. The presumptive *Salmonella* colony on the XLD and SS was selected and identified by using a series of biochemical tests.

Biochemical tests

All suspected non-lactose fermenting *Salmonella* colonies were picked from the nutrient agar and inoculated into the following biochemical tubes for identification: triple sugar iron (TSI) agar, Simmon's citrate agar, urea broth, MR-VP broth and incubated for 24 or 48 hours at 37°C. Colonies producing an alkaline slant (red) with acid (yellow color) butt on TSI with hydrogen sulphide production, negative for urea hydrolysis (red color), negative for tryptophan utilization (indole test) (yellow-brown ring), voges-proskauer after addition of alpha-naphthol and potassium hydroxide to the voges-proskauer broth (yellow-brown color) negative, methyl red test positive (if the culture changed to red after 4-5 methyl red reagent added) and positive for citrate utilization positive (changed to blue) were considered to be *Salmonella* [5].

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing for *Salmonella* isolates were carried out following the Kirby-Bauer disc-diffusion method on Mueller-Hinton agar (Oxoid CM0337 Basingstoke, England) as described in the Clinical and Laboratory Standards Institute (CLSI) guidelines (NCCLS, 2013). Each isolate was tested with their respective concentration (in brackets) for the following 10 different antibiotics (all from Oxoid); Chloramphenicol (3µg), Gentamycin (10µg), Streptomycin (10µg), Ciprofloxacin (5µg), Kanamycin (30µg), Nalidixic acid (30µg), Cefoxitin (30µg), Sulfamethazoletrimethoprim (25µg), Ampicillin (25µg) and Amoxicillin (10µg). From each isolate, four to five biochemically confirmed well isolated colonies grown on nutri-

ent agar 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 the turbidity was checked with 0.5 Macfarland. Subsequently, it was streaked on to the Muller-Hinton Agar. Then the antibiotic discs were placed on the medium and incubated at 37°C for 18 h, followed by measurement of zone of inhibition manually. Finally, the diameters of zone of inhibition were recorded to the nearest millimeter and the isolates were classified as susceptible, intermediate, or resistant as described elsewhere [5].

Data management and analysis

The data were entered in to Microsoft excel 2007 (Microsoft corporation, USA) and analyzed using the SPSS statistical software package version 20 (IBM SPSS Statistics, USA). The Chi-square test was utilized to assess significant differences in positivity of *Salmonella* isolates in sample types originated from dairy farms and abattoirs. The isolates were further screened for susceptibility to 10 different drugs and rated as susceptible, intermediated and resistant using frequency and proportions. A difference was taken as significant at a p-value less than 0.05 at 95% confidence interval for the variables analyzed by chi-square.

RESULTS

Frequency of isolation of *Salmonella*

Out of the total 182 (90 from abattoir and 92 dairy farm) samples collected for *Salmonella* bacteriological isolation, 11(6.04%) were positive. Of the 11 *Salmonella* isolates, the distribution of the isolates were 3(8.6%) in faeces from farm, 3(12.5%) in faeces from abattoir, 3(12.5%) in mesenteric lymph node swab, 1(20%) in pooled knife and 1(3.7%) in carcass swab. However, there was *Salmonella* isolate detected in other samples collected from both abattoir and dairy farms. No statistical difference between different sample types ($p > 0.05$) was observed for positivity of *Salmonella* (Table 1).

Antimicrobial susceptibility pattern of *Salmonella* from different sample types

All the eleven isolates of *Salmonella*, from farms and abattoir, were subjected to a panel of ten antimicrobials. The antimicrobial susceptibility pattern of the isolates indicated that all isolates were 100%, 81.8% and 81.8% sensitive to gentamycin, kanamycin and sulphamethazole trimethoprim respectively. On the other hand the isolates were 72.7%, 63.6%, and 54.5% resistant to streptomycin, cefoxitin and ampicillin (Table 2).

Multi drug resistance *Salmonella* isolated from different sample source

54.5% isolates were showed resistance for three or more of the antimicrobials tested. From these resistance isolates, most of them 72.7% and 63.6% showed resistance to streptomycin and cefoxitine followed by resistance to ampicillin, amoxicillin, chloramphenicol and sulphamethazoletrimethoprim 27.3%, 18% and 18% respectively (Table 3).

DISCUSSION

In the present study, the isolation of *Salmonella* from appar-

Table 1: Results of *Salmonella* isolated from different sample types.

Sample types	Total	Positive	Proportion (%)	χ^2	df	p-value
Feaces from farm	35	3	8.6	2.4	1	0.11
Feaces from abattoir	24	3	12.5			
Lymph node swab	24	3	12.5			
Carcass swab	27	1	3.7			
Pooled knife swab	5	1	20			
Bucket milk	36	0	0			
Tank milk	6	0	0			
Tank swab	4	0	0			
Bucket swab	5	0	0			
Hand swab from farm	6	0	0			
Hanging material swab	5	0	0			
Hand swab from abattoir	5	0	0			

Key: χ^2 = Chi square, df = degree of freedom

Table 2: Antimicrobial susceptibility pattern of *Salmonella* from different sample types.

Types of drug	No. of susceptibility (%)	No. of intermediate (%)	No. Resistance (%)
AML	3(27.27)	5(45.5)	3(27.3)
AMP	0(0)	5(45.5)	6(54.5)
CN	11(100)	0(0)	0(0)
CP	3(27.3)	8(72.7)	0(0)
C	8(72.7)	1(9)	2(18)
FOX	1(9)	3(27.3)	7(63.6)
K	9(81.8)	2(18)	0(0)
NA	8(72.7)	3(27.3)	0(0)
S	2(18)	1(9)	8(72.7)
SXT	9(81.8)	0(0)	2(18)

*Key: AML= Amoxicillin, AMP= Ampicillin, CN= Gentamycin, CP= Ciproflaxin, C= Chloramphenicol, FOX= Cefoxitin, K= Kanamycin, NA= Nalidixic acid, S= Streptomycin, SXT= Sulphamethazole-trimethoprim

Table 3: Multi drug resistance *Salmonella* isolated from different sample source.

No. of antimicrobial resistance	Antimicrobial resistance pattern	No. of <i>Salmonella</i> isolates (%)
One	AMP, S	2(18)
Two	FOX,S S, FOX	2(18)
Three	AMP, S, SXT AMP, FOX, S C, FOX, S	3(27.3)
Four	AMP, AML,FOX, SXT AMP, AML, FOX, S	2(18)
Five	AMP, AML, FOX,C, S	1(9)
Overall		10(90.3)

N.B. Multidrug resistance (MDR) is the resistance to ≥ 3 antimicrobials

ently healthy lactating dairy cattle farms was 3.33%. The current study report was higher than others in that 1.25% from dairy cattle feaces [18] and 2.1% from preweaned dairy heifers [19]. The present findings are lower than the reports of [20,21] which indicated that the isolation of 10.76% and 11%, respectively. In contrast, higher isolation of *Salmonella* 34.2% [22] and 44% from dairy cattle feaces [23] were reported. The difference in reported isolation could be associated with sampling plan and procedures, bacteriological technique employed in detecting *Salmonella* or difference in occurrence and distribution in the study populations regardless of test samples and methods of detection [24].

The isolation and subsequent identification of *Salmonella* depends not only on the quality of the sample but also on the culture medium and growth characteristic of the serovars, particularly those adapted to host specific [25] *Salmonella* is more frequent in dairy herds than beef herds, mixed dairy and beef herds and calve herds, large herds and confinement [26]. It is also known that overcrowding, poor hygienic condition and large herds exacerbate the distribution of *Salmonella*. In addition, *Salmonella* on dairy operations include addition of replacement animals without testing them, failure to routinely test feed components for *Salmonella*, poor control of wild birds and rodents, inadequate sanitation in calving and calf-rearing area will increase feco-oral

transmission of *Salmonella* pathogen [27]. It is well documented that, when animals are staved, *Salmonella* can survive and multiply in the rumen.

In the present study, the proportion of *Salmonella* isolated from fecal specimen of apparently healthy slaughtered animals in the study area was 8.7%. There were reports 7.1% [28] of *Salmonella* isolate from feces of apparently healthy slaughtered animals in the abattoirs. However, the proportion we found was lower as compared to 14% [29]. This could be as a result of longer time that the cattle stay in the lairage before slaughter. It has been shown that a decrease in the daily feed intake enhanced the growth of *Salmonella* in the rumen and fecal excretion by carrier animals [29]. Higher proportion of *Salmonella* isolates from different reports might have been caused by the resumption of feeding after transportation to slaughter house or due to cross contamination through feeding and watering troughs during 24 to 72 hours when animals stayed in the lairage. The 12.5% of *Salmonella* proportion in mesenteric lymph nodes in the investigation was higher than previous reports of 2.1% [30] and 0.9% [28] from Addis Ababa abattoir and Faculty of Veterinary Medicine abattoir at Bishoftu, respectively. The reports of [31] indicated the proportion of *Salmonella* in the mesenteric lymph node 7.24% in cattle which was lower than the present study. However, our mesenteric lymph node proportion was much lower than the 57.1% [32] and 30% [33] from two different commercial abattoirs in Australia. The relatively lower *Salmonella* proportion in this study as compared to those from the latter two studies could be attributed to the differences in animal husbandry where animals live in a confined environment and require frequent cleaning [34].

The proportion of *Salmonella* from carcass in our study was 3.5%. This was in agreement with the 2.8% and 3.1% *Salmonella* proportion from abdominal muscles and diaphragmatic muscles, respectively, from cattle slaughtered in small abattoir at Faculty of Veterinary Medicine Debra Zeit [28]. It was also lower than the 7.6% report from the Republic of Ireland [29]. These differences could be attributed to the differences in abattoir facilities, sampling techniques, number of animals' slaughtered and level of hygiene maintained by the abattoirs. Our carcass proportion of *Salmonella* was in agreement with 2% carcass contamination before chilling from an abattoir in Australia [35] and 1.3% of beef carcass contamination in USA [36]. The similarities and differences in carcass contamination levels have to be taken with caution because of the differences in which the studies were conducted. For example, the carcasses contamination rates in the latter two studies were obtained after a number of decontamination measures were taken, to reduce the bacterial load of carcasses, while no decontamination measures were under taken in the current study, expecting cases where there were accidental spillages of gut contents, during which it was washed with tap water by sprinkling with a rubber hose. The presence of even small number of *Salmonella* in carcass meat and edible offal may lead to heavy contamination of minced meat and sausages [37]. Therefore, as the slaughter house produces minced beef for a number of local super markets further amplification of *Salmonella* could occur, becoming a public health risk. In the present study, 20% *Salmonella* proportion obtained from the pooled knife swab was higher than the 7.4% proportion of *Salmonella* isolated by [2], from evis-

cerating knife swab in Modjo export abattoir. In addition, cutting knives, sows, hoes, meat grinders, cutting boards and storage utensils, which are not properly cleaned and disinfected, serve as means of cross-contamination for *Salmonella* from contaminated meat to clean meats [38,3]. Therefore, in our study the high proportion of *Salmonella* obtained in the study area due to using the same knives for evisceration and dressing which exacerbate the contamination of carcasses. All of the isolates obtained during study period (n =11) were tested for ten different antimicrobial that were available in the market. The susceptibility ranges from 0 up to 100% antimicrobials. The most common test pattern susceptibility was for Gentamycin [3]. reported that isolates of *Salmonella* from food items and personnel from Addis Ababa were resistant to commonly used antibiotics including streptomycin, ampicillin and tetracycline. The results of present study also suggests the resistance of *Salmonella* isolates to commonly used antimicrobials including streptomycin, cefoxitin, ampicillin, amoxicillin, chloramphenicol, and sulfamethazoletrimatoprim with the resistance rate of 72.7%, 63.6%, 54.5%, 27.3%, 18% and 18%, respectively. In the present study, the resistance of streptomycin was 72.7%. These results were higher than 11.1% [39,40,41]. Of the total tested isolates; 72.7%, 63.6%, 54.5%, 27.3%, 18% and 18% of isolates were resistant to streptomycin, cefoxitin, ampicillin, amoxicillin, chloramphenicol, and sulfamethoxazoletrimethm, respectively. However, all the isolates were susceptible to gentamycin. Six isolates were resistant at least for three or more antimicrobials. Ten of the total isolates were resistant to one or more of the tested antimicrobials; 54.5% were multiple antimicrobial resistant while the rest were resistant to single antimicrobial. This finding is in contrast to [3] who reported 25% antimicrobial resistant *Salmonella* isolates from cottage cheese. Detection of antimicrobial resistant *Salmonella* might be associated with their frequent usage both in livestock and public health sectors as these antimicrobials are relatively cheaper and commonly available. The effectiveness of Gentamycin this study might be due to the difference in frequency of usage among the available antimicrobials, the nature of drugs, and their interaction with the bacteria. Different individuals reported antimicrobial resistant *Salmonella* isolates in previous studies from Ethiopia [42,43,6].

CONCLUSIONS

The occurrence of *Salmonella* in dairy farms and apparently healthy slaughtered animals in Adama town is found to be 3.4% and 8.8%, respectively. This result is significantly higher to be a potential source of food borne salmonellosis putting human health at risk via food chain. High proportion (54.5%) of *Salmonella* isolates were resistant to three or more of the antimicrobials that are commonly used in the veterinary and public health set up. This may cause difficulties in the treatment of human clinical cases and other bacterial disease as different bacteria species exchange the drug resistant gene under field conditions. The currents study indicated the necessity of a further investigation on the prevalence and antimicrobial susceptibility pattern of *Salmonella*, by considering it as a potential food borne pathogen.

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