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Isolation, Identification and Antimicrobial *Susceptibility* Profile of *Salmonella* Isolates from Abattoir and Dairy Farms in and Around Holeta Town, Oromia, Ethiopia

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Abstract

A cross sectional study was carried out from February 2014 to April 2014 to isolate, identify and assess the antimicrobial resistance profile of *Salmonella* from selected dairy farms and abattoir in Holeta town. A total of 232 samples were collected; 126 samples were from dairy farms (rectal feces, udder milk, pooled milkers hand swab, tank milk, tank swabs, and bucket swabs) while 106 samples from abattoir (carcass swab, rectal feces, pooled knives swab, pooled hanging materials and hand swab). The isolation and identification of *Salmonella* was carried out according to the techniques recommended by the international organization for standardization (ISO 6579, 2012). The overall proportion of *Salmonella* positive was found to be 5.6% (13/232). Of a total sample, 9.1%, 2.2%, 33.3%, 16.7%, 16.7 % and 6.7% was *Salmonella* positive observed from carcass swab, feces, pooled knives swab, poled hand swab, pooled hanging materials swab and udder milk, respectively. However, there was no statistically significant difference ($p > 0.079$) observed in the proportion of *Salmonella* positive isolates among the sources. The antimicrobial susceptibility profile of all isolates wasassessed against ten antimicrobials by disk diffusion technique; almost all isolates were resistant to one or more of the tested antimicrobials. Of all isolates 53.2% were multi drug resistant (MDR). 76.9%, 69.1%, and 38.5% of the isolates were resistant to streptomycin, chloramphenicol and ampicillin, respectively. However, the majority of the isolates were susceptible to ciprofloxacin, and gentamycin. From this study, it is concluded that dairy farms and abattoir are a potential source of *Salmonella* infection with antimicrobial resistance. This is a significant threat to public health particularly to those who have direct or indirect contact to animal and animal products. Furthermore, hygienic management of dairy farms, abattoirs and prudent use of antimicrobials is recommended.

INTRODUCTION

Food borne diseases are a continuing challenge to human health worldwide. Over the past two decades, the epidemiology of food borne diseases has changed rapidly as a consequence of changes in the social environment and the ability of pathogens to adapt to new niches. Food-borne pathogens are the causes of illness and death in developed and developing countries, which resulting in the loss of labor force which could have contributed in the economic growth [1]. Changes in eating habits, mass catering, unsafe food storage conditions and poor hygiene practices are major contributing factors to food associated illnesses. The risk of the transmission of zoonotic infections is also associated with contaminated feces, egg, meat, milk and milk products [2]. Salmonellosis is considered to be one of the most foods borne illnesses in humans, with globally and is economically important disease of all animals' species. The natural habitat of *Salmonella* is the intestinal tract of human and other animals. Both water and foods of animal origin have been identified as vehicles for transmission of the organism. Approximately 95% of cases of human Salmonellosis are associated with the consumption of contaminated animal's products such as meat, poultry, eggs, milk, seafood, and fresh produce [3]. *Salmonella* species are leading causes of acute gastroenteritis in several countries and remains an important public health problem worldwide, particularly in the developing countries. It is the most common food borne disease in developing countries, although incidence rates vary according to the country. The fecal wastes from infected animals and humans are important sources of bacterial contamination of the environment and the food chain. In Ethiopia, as in other developing countries, it is difficult to evaluate the burden of Salmonellosis because of the limited scope of studies and lack of

coordinated epidemiological surveillance systems. In addition, under-reporting of cases and the presence of other diseases considered to be of high priority may have overshadowed the problem of Salmonellosis. The real situation of antibiotic resistance is also not clear since *Salmonella* are not routinely cultured and their resistance to antibiotics cannot be tested. As in a developed country, however, to control the spread of Salmonellosis, surveillance for *Salmonella* serovars and the assessment of antimicrobial susceptibility is essential [1,4,].

The use of antibiotics in human and veterinary medicine has resulted in a spectacular decrease in the mortality rate of infectious diseases. However, this contribution to therapeutics has not been without disadvantages, one of the most outstanding is the evolution of antibiotic resistance. This has resulted in the dissemination of resistance genes to sensitive species and the emergence of new resistance determinants [5]. Antimicrobial resistance is a global public health problem. Although all countries are affected, the extent of the problem in the developing nations is unknown. 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 foods product [6-8].

It is possible to isolate and identify *Salmonella* either from tissues collected aseptically at necropsy or from feces, milk, blood, rectal swabs or environmental samples. When infection of the reproductive organs or concepts occurs, it is necessary to culture fetal stomach contents, placenta and vaginal swabs and, in the case of poultry, egg contents. However, Salmonellosis is particularly difficult to determine in clinically normal carrier animals. It represents an important prerequisite for the detection of the source of infection and the route of transmission [9]. Various biochemical and serological tests can be applied to the pure culture to provide a definitive confirmation of an isolated strain [10].

Control and prevention of Salmonellosis is difficult because of its distribution in nature in all types of climate and harbored in human and many various animal species. Furthermore, compared with other Gram-negative organisms, *Salmonella* are relatively resistant to different environmental factors therefore, a periodic surveillance of the level of *Salmonella* contamination in the different food animals, food products and environments is necessary to control the spread of the pathogen and infection in human. Therefore, the objective of the present study is to Isolate, identify and determine the level of antimicrobial resistance pattern of isolated *Salmonella* from abattoir and dairy farms in and around Holeta town.

MATERIALS AND METHODS

Study area

The study was carried out in Holeta town from February 2014 to April 2014.Holeta is located at a distance of 44km from Addis Ababa. It has a latitude and longitude of 9°3′N, 38°3′E and an altitude of 2391 meters above sea level (m.a.s.l). The area is characterized by mild subtropical weather; with minimum and maximum annul temperature of 6.3°C and 22.1°C, respectively and with an average of 14.5°C. The area also experiences a bimodal rainfall pattern with along rainy season extending from July to September while the short rainy season extends from March to April. The minimum and maximum annual rainfall of the area ranges from 834mm and 1300mm and with an average 1067mm. The district has an estimated total population of 25,593, whom 12,605 were men and 12,988 women. Agriculture is the major source of economy and it includes mainly the cultivation of *teff*, barley and cattle rearing.

Study design, sample size determination and study populations

A cross-sectional study was carried out from February 2014 to April 2014 with the aim of isolation, identification and assessment of the antimicrobial resistance profile of *Salmonella* isolates. The sample size was calculated according to the formula given by (Thrusfield, 2005), based on expected prevalence of *E. coli* O157 in goat meat and feces [11], where the confidence level was 95% and the precision was 5%. Thus, the required sample size was 73; however 232 samples were collected in order to maximize the precision of the study. The study populations were apparently healthy cattle for slaughter in Holeta municipality abattoir, lactating dairy cows, equipment as well as personnel's' hand. The study samples were selected randomly from cattle for slaughter, personnel's hand, and equipment and lactating cows.

Sample collection and transportation

A total of 232 samples were collected randomly from abattoir and dairy farms. Of which, 106 samples were from abattoir (44 carcass swabs, 6 pooled hanging material swabs, 6 pooled knives swab, 6 pooled evisceration hand swabs and 44 rectal feces) and 126 from dairy farms (45 udder milk, 9 tank milk, 9 milkers' hand swabs, 9 bucket swabs, 9 tank swabs and 45 rectal feces) samples were collected. The samples were collected in a sterile container with buffered peptone water and each sample was labeled legibly and accomplished by the necessary identification information which includes the date of sampling, type of sample, source of sample and immediately transport using ice box to the microbiology laboratory of the college of veterinary medicine and agriculture of Addis Ababa University for microbiological analysis.

Bacteriological examination

The study was conducted by conventional methods for the detection of *Salmonella* following the standard guidelines from (ISO 6579, 2012).

Biochemical tests

All suspected *Salmonella* colonies were picked from the nutrient agar and inoculated into the following biochemical test tubes for identification: Triple sugar iron (TSI) agar, Simmons's citrate agar, Urea agar, Tryptone soya broth, and Methyl Red Voges Proskauer (MR-VP) broth incubated for 24 or 48 hours at 37°C. Colonies producing an alkaline slant with acid (yellow color) butt on TSI with hydrogen sulfide production, negative for Urea hydrolysis, negative for Indole test utilization (yellow-brown ring), positive for Methyl red (red color), negative for Voges-Proskauer,

and positive for Citrate utilization was consider to be *Salmonella* positive [12].

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, Basing stoke, England) according to the National Committee for Clinical Laboratory Standards (NCCLS, 2012). The isolates were tested with their respective concentration (in brackets) for the following antibiotics; ampicilin (10µg), amoxacilin/C (20µg), chloramphenicol (30µg), gentamycin (10µg), streptomycin (10µg), ciprofloxacin (5µg), kanamycin (30µg), nalidixic acid (30µg), cefotrixin (30µg) and sulphamethazole-trimethoprim (23.5µg) all from Oxoid. From each isolate, four to six biochemically confirmed well-isolated colonies grown on nutrient agar were transferred into tubes containing 5ml of Tryptone soya broth (Oxoid, England). The broth culture was incubated at 37°C for 4-6 hr until it achieved the 0.5 McFarland turbidity standards. Sterile cotton swab was dipped into the suspension and the bacteria were swabbed uniformly over the surface of Muller-Hinton agar plate (Oxiod, CM 0337, and Basingstoke, England). The plates were held at room temperature for 15-30 min to allow drying. Antibiotic discs with known concentration of antimicrobials were placed and the plates were incubated for 18-24 hr at 37°C. The bacterial characteristics were the main criteria used to select the antimicrobial agents. Moreover, selection was also based on their mechanisms of action and availability. *Salmonella* ATCC 14028 were used as reference strains for quality control of the antibiotics used. The diameters of zone of inhibition were recorded to the nearest millimeter and classified as resistant, intermediate, or susceptible according to published interpretive chart (NCCLS, 2012).

Data analysis

Data was analyzed by using SPSS version 20 computer software (SPSS 20.0 Command Syntax Reference, SPSS Inc. Chicago, 2013). The Chi-square test was utilized to assess significant differences of *Salmonella* status in the type and source of samples.

RESULTS

A total of 232 samples originating from abattoir and dairy farms were analyzed by conventional culture method for detection of *Salmonella*. Bacteriological examination was conducted on 106 samples from abattoir (carcass swabs, pooled hanging line swabs, rectal feces, pooled knives swab, and eviscerate personnel hand swabs) and 126 samples from selective dairy farms (udder milk, bucket swabs, tank milk, tank swabs, pooled milkers' hand swab, and rectal feces). Out of a total samples analyzed, 13(5.6%) *Salmonella* positive were isolated from abattoir and dairy farms and there is no statistically significant difference observed in the isolation of *Salmonella* from abattoir and dairy farms (x^2=3.076, p=0.0790).

From a total of 126 samples collected from dairy farms, 4 (4.4%) were positive for *Salmonella* isolates (from milk and feces). Of these, 3/5(6.7%) and 1/45(2.2%) were found to be *Salmonella* positive from udder milk and fecal samples, respectively (Table 1).

Out of a total of 106 samples collected from abattoir, 9(8.5%) were found to be positive for *Salmonella* species, 4(9.1 %) from carcass swab, 1/6(16.7%) from eviscerating hand swab, 2/6(33.3%) from pooled knives swab, 1/6(16.7%) from pooled hanging line swab, 1/44(2.3%) from rectal content samples, were found positive for *Salmonella* (Table 1). None of *Salmonella* was isolated, samples collected from pooled tank swabs, bucket swabs, tank milk, and milkers' hand swab.

Antimicrobial resistance

All of the 13 isolates of *Salmonella* from dairy farms (n=4) and abattoir (n=9) were subjected to a panel of 10 antimicrobials. The antimicrobial susceptibility pattern of the isolates indicated that the isolates were 76.9%, 69.1% and 38.5% resistant to streptomycin, chloramphenicol and ampicillin respectively. On the other hand, the isolates were 100%, 8%, 76.9%, and 69.2% sensitive to ciprofloxacin, gentamycin, cefotrixicin, and amoxicillin/C, kanamycin, and nalidixic acid, respectively (Table 2). Most of the isolates from abattoir were resistant to streptomycin and chloramphenicol, but highly sensitive tociprofloxacin and gentamycin.

Salmonella isolated from milk samples showed 100% resistance to chloramphenicol and streptomycin while 100% sensitive to ciprofloxacin and gentamycin. Faecal isolate of *Salmonella* from cow showed 100% sensitivity to ciprofloxacin and gentamycin but resistance to most of the tested antimicrobials. Among the antimicrobials which *Salmonella* isolates were tested, the most frequent resistance encountered was for streptomycin and chloramphenicol in which ten (76.9%) and nine (69.2%) were observed respectively.

A total of 7(53.8%) multiple drug resistance (MDR) pattern was observed. The highest/frequent MDR noted were/Amp/Cr, 3/13(23.1%). The maximum MDR registered was resistance to eight antibiotics with the combination of S/K/C/Aml/Amp/SXT/Fox /Na being more frequent (Table 3). In general, 69.8% *Salmonella* isolates showed resistance to against two or more antimicrobials tested and all of the total isolates except for ciprofloxacin were resistant to one or more of the tested antimicrobials.

DISCUSSION

In the present study, the overall proportion of *Salmonella* positive was 5.6% (13/232). Out of a total of 106 samples from abattoir and 126 from dairy farms examined for bacteriological status of *Salmonella*, 8.5% (9/106) and 3.2%(4/126) were found to be *Salmonella* positive, respectively. Of carcass swab samples analyzed, 9.09% were positive for *Salmonella* and it is supported with the findings of D'Aoust (1989) who reported that the contamination rate of beef carcass with *Salmonella* varies from 0.2 to 21.5%. The present finding was lower than report of Aftab et al., (2012) 12.5-25% was positive for *Salmonella* from carcass in Pakistan. But higher than the finding of Alemayehu et al, (2003) and report by [13], which ranged from 0.95 to 3.8% and 6.5%, in cattle slaughter at Addis Ababa, Faculty of veterinary medicine small slaughterhouse in Debre Zeit, and in Ethiopia respectively. This higher *Salmonella* status could be as a result of longer time that the cattle stay in the lairage before slaughter, poor hygiene of equipment and workers in abattoir, improper evisceration of GIT,

Table 1: Proportion of *Salmonella* species isolated from abattoir and dairy farms.

Source of sample	Sample of type	No of samples	Positive (%)
Abattoir	Carcass swab	44	4(9.0)
	Pooled knives swab	6	2(33.3)
	Personnel hand swab	6	1(16.6)
	Pooled hanging swab	6	1(16.6)
	Rectal feces	44	1(2.27)
Dairy Farms	Udder milk	45	3(6.67)
	Rectal feces	45	1(2.22)
	Pooled tank swab	9	-
	Bucket swab	9	-
	Milkers'hand swab	9	-
	Tank milk	9	-
	Total	232	13

Table 2: Antimicrobial Sensitivity test results of *Salmonella* isolates from abattoir and dairy farms.

Types of antimicrobials	Disc concentration(µg)	Number of isolate	Resistant (%)	Intermediate (%)	Susceptible (%)
Ampicilin	30	13	5(38.46)	1(7.62)	7(53.84)
Amoxacilin/CL	20	13	3(23.07)	-	10(76.9)
Chloramphenico	30	13	9(69.13)	-	4(30.76)
Ciprofloxicin	5	13	-	-	13(100)
Sulpham/trim	23.75	13	4(30.76)	1(7.62)	8(61.5)
Ceftrixicin	30	13	3(23.07)	-	10(76.9)
Kanamycin	30	13	4(30.76)	-	9(69.23)
Streptomycin	10	13	10(76.9)	-	3(23.07)
Nalidixic acid	30	13	4(30.76)	-	9(69.23)
Gentamycin	10	13	2(15.98)	-	11(84.65)
Total	**130**				

Table 3: Multiple antimicrobials resistance pattern of isolated *Salmonella.*

Number of antimicrobial resistance	Antimicrobial resistance pattern	Number of isolates (%)
Zero	-	1(7.69)
One	Cn (1),Cr (1),S (1)	3(23)
Two	Cr/S	2(15.38)
Three	-	0(0)
Four	Cr/S/K/Fox	1(7.69)
	Cr/S/K/Na	1(7.69)
	Amp/S/Cn/Aml	1(7.69)
Five	Amp/Cr/S/SXT/Fox	1(7.69)
	Amp/Cr/S/Na/SXT	1(7.69)
Seven	Amp /Cr/S/k/Na/SxT/Aml	1(7.69)
Eight	Amp/Cr/S/K/Na/Sxt/Fox/Amp	1(7.69)
Total		**13(100%)**

Multi drug resistance (MDR) ≥ 3drug/isolates resistance 7/13(53.84%)
Abbreviations: Cn=Gentamycin, Cr=chloramphenicol, S=Streptomycin, K=Kanamycin, Amp=Ampicilin Na=Nalidixic acid, Aml=Amoxacilin, Fox=Cefotrixicin, Sxt=Sulphomethazole/Trimoprtin

multiple incisions of lymph nodes (Mesenteric) during slaughter and cross contamination. Amongst the microbes, *Salmonella* most frequently present on animal body coat and feces and transferred to carcasses during slaughtering and cause severe damages to human health if consumed and it causes food poisoning in the world [14].

In this study, 33.3% of *Salmonella* positive samples were obtained from the pooled eviscerating knives swabs. This study was in contrast with 5% prevalence of evisceration knives study in Queensland, Australia [15], 5% prevalence on killing knives in poultry slaughterhouse in Iraq [16], 7.4% in Modjo abattoir [17] and reports of 26.7% and 10% from Botswana abattoir A and B [18], respectively. An attempt was also made to examine *Salmonella* on pots, hand nails and hands in abattoir in Queensland, in which *Salmonella* were isolated from the hands of workers in all stages along the slaughtering line with 30% on hands of workers in evisceration [19]. In this study, pooled knives swab (16.6%)

indicated that it is higher than the findings of [18] (8.9%) and 9% [20] who reported from abattoir workers' hands in Modjo and Debre Zeit, respectively. Higher prevalence in this study might be as result of cross contact with hide, feces and internal organ during evisceration.

Salmonella are typically intestinal pathogens where infected animals may excrete the organism in their feces, especially during stresses contain the environment and transfer the infection to others remaining clinically normal. The carrier animals bear the *Salmonella* in their mesenteric lymph nodes, liver, spleen and gall bladder [21]. [22] examined 47 pooled fecal samples and reported 10.6% *Salmonella* positive, but in our findings, out of 45 samples, (2.22%) were found *Salmonella* positive. Relatively high incidence rate of *Salmonella* isolation reported by Nyeleti (1999) [22] might attributed to the stress of long transportation on foot and commingling of cattle from different source at the lairage as well as the low hygiene of abattoir and starvation. 126 samples were collected from dairy farm for bacteriological analyzed and 3.17% (4/126) were found positive for salmonella. Of sample collected, 6.67 % (3/45) and 2.22% were *Salmonella* positive from udder milk and rectal fecal sample respectively. Hence lactating cows could be potential sources of *Salmonella* infection for individuals working in dairy farms and for the community at large. The present result from udder milk was higher than report from Iran, 4% [23], but in contrast with 20% [24], 16% were *Salmonella* positive reports from raw milk at Jimma, Sebeta and Cameron respectively.

In this study, all of the 13 *Salmonella* isolates were tested against 10 antimicrobials drugs and 92.3% were found to be resistant to one or more antimicrobial drugs. High percentage of multiple resistance *Salmonella* isolates of cattle origin to the commonly used antimicrobial observed in this study could pose significant public health risks. Anti-microbial resistant *Salmonella* isolates from animals and human source have been reported in Ethiopia [14,27]. In present study, 69.2% of the *Salmonella* isolates from abattoir and dairy farm were resistant to two or more antimicrobials for drugs commonly used to treat bacterial infections in domestic animals and human being in Ethiopia. The result indicates that 53.84% of isolates were Multi-drug resistance (MDR) and the finding of present study are supported with the previous studies of 52% [25], but higher than Zewdu (2004) report 25% resistant isolates from cottage cheese and [26] report 50% resistant isolates from dairy product. But lower than81.75% reports from Sebeta which indicated that antimicrobial resistance of *Salmonella* isolates in sub-Saharan Africa including Ethiopia is generally high which is attributed to the misuse of antimicrobials both in veterinary and public health practice. Detection of antimicrobials resistant *Salmonell*a might be associated with their frequent usage both in livestock and public health sectors as these antimicrobials are relatively cheaper and commonly available [27]. Anti-microbial resistance is currently the greatest challenge to the effective treatment of infections globally [28]. For instance, more than 80% of food poisoning bacteria such as *Salmonella* are reported as antibiotic resistant to at least one type of antimicrobial and more than 50% as resistant to two or more. Globally, the three main causes of antimicrobial resistance have been identified as use of antimicrobial agents in agriculture, over-prescribing by physicians and misuse by patients [28,29]. The result of the current research also indicated that resistance of *Salmonella* isolates to commonly used antimicrobials including Streptomycin, Chloramphenicol, Ampicillin, Kanamycin, with resistance rate of 76.9%, 69.23%, 38.46%, and 30.23% respectively. This result is comparable with reports of [1] that isolates *Salmonella* from food handlers in Addis Ababa University Cafeteria and Zewdu and Cornelius (2009) [30] report of *Salmonella* from food items and personnel from Addis Ababa.

Ciprofloxacin and Gentamycin showed a good antimicrobial, activity against both abattoir and dairy farm *Salmonella* isolates. This is also comparable with the result reported by [28] from Addis Ababa among lactating cows, [26] from Addis Ababa among dairy product, [1] from food handlers in Addis Ababa university cafeteria, [32] from Nigeria among human isolates and reports by Mollaet al., (2006) from central part of Ethiopia among isolates of sheep and goat and Abraham et *al.,* (2011) from Sebeta among raw milk. Though no data has indicated this, the effectiveness of such drugs like ciprofloxacin may be because they are not widely used in countries like Ethiopia and other African countries for animal's treatment. In our study, isolated *Salmonella* 38.46% (5/13) was resistant to ampicillin. This finding is disagree with previous reports from Addis Ababa [28], Bahir Dar [31], from Nigeria [32] and from Addis Ababa [26] which reported 100%, 100%, over 90% and 50% resistance to Ampicillin, respectively. This could may due to frequent use and easily available in everywhere in the world.

CONCLUSION

Salmonella are the major pathogenic bacteria in humans as well as in animals. It is the most common food borne disease in both developing and developed countries, although incidence rates vary according to the country. The fecal wastes from infected animals and humans are important sources of bacterial contamination of the environment and the food chain. High proportion (92.3%) of *Salmonella* isolates were resistant to one or more of the antimicrobials that are commonly used in the veterinary and public health set up. This may pose difficulties in the treatment of human clinical cases and other bacterial diseases, so wise use of antimicrobials must be practiced to combat the ever increasing situation of antimicrobial resistance.

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