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Isolation, Identification and Antimicrobial Susceptibility Profile of *Salmonella* Isolates from Abattoir and Selected Dairy Farms of Addis Ababa City, Ethiopia

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Abstract: The study was conducted to isolate, identify and investigate theantibiotic susceptibility pattern of Salmonella from abattoir and dairy farms of Addis Ababa, Ethiopia. Across sectional design study was undertaken over a 5-month period between January 2017 and May 2017 on total of 201 samples (108 samples from slaughter house and 93 from dairy farms). Results showed that theoverall proportion of salmonellae was 7.5% (15/201) (abattoir n=11, 10.2% and dairy farms n=4, 4.3%). The antimicrobial resistance profiles of 15 salmonella isolates with 10 antimicrobials showed that about 86.9, 73.3, 67.7, 60 and 53.3% were resistant to ampicillin, kanamycin, nalixidic acid, amoxicillin and cefoxitin respectively. On the other hand the isolates were, 100, 93.3, 73, 60 and 53.3% sensitive to ciprofloxacin, gentamycin, streptomycin, sulphamethoxazole-trimethoprim and chloramphenicol respectively. Among the isolates 86.7% of both abattoir and dairy farm isolates were showed resistance for two and more of the antimicrobials tested. Higher proportion of *Salmonella* was isolated from abattoir than dairy farms. High proportion of *Salmonella* isolates developed resistance to commonly prescribed antimicrobials and this may pose a considerable risk in the treatment of clinical cases. So, the currents study indicated wise use of antimicrobials must be practiced to combat the ever increasing situation of antimicrobial resistance and the necessity of a further investigation on the prevalence and antimicrobial susceptibility pattern of *Salmonella*.

Key words: Abattoir · Antimicrobial · Dairy Farms · Food · Identification · Isolation andSalmonella

INTRODUCTION

The safety of food of animal origin is one of the greatest issues in all over the world. Food provision is one of the contributions from animals to human; however, unsafe food causes many acute and lifelong diseases. Food borne diseases and threats to food safety constitute a growing public health problem. Therefore, ensuring safety of products of animal origin from primary production to the consumer must be a priority for public health and veterinary authorities as well as for the food industry [1]. Food contamination with the pathogen can occur at multiple points along food chain (farm to plate system), including production, processing, distribution, retail marketing and handling or preparation [2]. Food borne pathogens, such as *Salmonella, Shigella* and *E. coli,* can cause human diseases and meat is one of the vehicles for human infection [3].

Salmonellae are facultatively anaerobic, Gram-negative rods belonging to the family Enterobacteriaceae. Currently, the genus is divided into two species, *Salmonella enterica* and *Salmonella bongori* [4]. *Salmonella* enterica is divided into six subspecies (*enterica, salamae, arizonae, diarizonae, houtenae* and *indica*), each of which has several serovars or serotypes. Nowadays, more than 2, 500 serotypes are known and most of them (almost 1, 500) belong to subspecies enterica [2]. Although members of this genus are motile by peritrichous flagella, non flagellatedvariants and non-motilestrains resulting from dysfunctional flagella do occur [5]. *Salmonella* grows between 8 and 45°C

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(optimally at 37°C) and at a pH of 4 to 9. A temperature higher than 70°C rapidly kills them. Pasteurization at 71.1°C for 15 seconds is sufficient to destroy *Salmonella* in milk [6].

Salmonella is the major pathogenic bacteria in humans as well as in animals. *Salmonella* species are leading causes of acute gastroenteritis in several countries and *Salmonella* remains an important public health problem worldwide, particularly in the developing countries [7]. Salmonellosis is the most common food borne disease in both developing and developed countries, although incidence rates vary according to the country [8]. The fecal wastes from infected animals and humans are important sources of bacterial contamination of the environment and the food chain [9].

Foods of animal origin, particularly meat, poultry and in some instances, unpasteurized milk products are considered to be the primary sources of human salmonellosis [6]. Most of these food products, e.g. beef, mutton and poultry, become contaminated during slaughter and processing, from the gut contents of healthy excreting animals. In the same way, all food that is produced or processed in a contaminated environment may become contaminated with salmonellae and be responsible for outbreaks or separate cases of disease as a result of faults in transport, storage, or preparation [5].

The epidemiology of food borne problems like salmonellosis is complex and expected to vary with change in the pathogens themselves, industrialization, urbanization and change of lifestyles, knowledge, belief and practices of food handlers &consumers, demographic changes (increased susceptible population), international travel &migration, international trade in food, animal feed &in animals, poverty and lack of safe food preparation facilities [10].

In developing countries a rapidly growing industry of intensive animal production is accompanying the process of urbanization with all its environmental and behavioral changes favorable for *Salmonella* to prevail [10]. Most food industries in developing countries are not well aware of food safety issues and knowledge of modern technologies, Good Manufacturing Practices (GMP), hygiene, Hazard Analysis Critical Control Point (HACCP) system and quality control is often limited or absent. Cold storage facilities are inadequate and quality of water used for food processing may not be suitable. The vast numbers of laborers that handle food in factories, as well as on farms, are illiterate and untrained [11].

The extensive use of antimicrobials in human and animals has led to an increase in bacterial multidrug resistance among several bacterial strains. This phenomenon of multiple resistances represents a worldwide problem both for veterinary and public health sectors. Bacterial resistance is observed especially when the antibiotics are abundantly used and that the bacteria can be transmitted easily between the individuals. Various antimicrobials in intensively managed food animals including chickens are often administered through feed or drinking water either for therapy, prophylaxis or growth promotion. *Salmonella* species is one of the most frequently isolated bacteria in food of animal origin. The increasing single and multiple antimicrobial-resistant *Salmonella* strains isolated from human cases of salmonellosis have been associated with widespread use of antimicrobial agents in food animal. This may represent a public health risk by transfer of resistant *Salmonella* strains to humans through the consumption of contaminated food and food products [12]

Different studies conducted in Ethiopia indicated considerable prevalence and antimicrobial resistance of *Salmonella* both in veterinary [13-17] and public set ups [18-20].

Objectives:

- To isolate and identify *Salmonella* from abattoir and dairy farms of Addis Ababa.
- To investigate the antibiotic susceptibility pattern of Salmonella isolates derived from abattoir and dairy farms.

MATERIALS AND METHODS

Sampling Methods and Sample Size Determination: The study was conducted from January, 2017 up to May, 2017 in Addis Ababa and the animals were selected by using simple random sampling method.

Sample size was determined using prevalence rate of 7.1% from previous studies [24] at 5% desired absolute precision and 95% confidence interval using the formula recommended by Thrusfield [21].

$$n = \frac{1.96^2 Pexp\ (1\text{-}Pexp)}{d^2}$$

where Pexp = expected prevalence; d= absolute precision; n =sample size. The estimated sample size will be….

Based on the above formula the calculated sample size was 201

Sample Collection and Transportation: A total of 201 samples (108 from abattoir and 93 from dairy farms) were collected. The abattoir samples consisted of (n=30) feces from the rectum of animals and swabs of (n=60) carcass and (n=18) pooled carcass incontact materials of the abattoir (knife, butchery hand and hanging material). The samples from dairy farms included (n=33) udder milk, (n=32) fresh faecal samples collected directly from the rectum of healthy cow and (n=28) swabs from milk incontact surface of the farm (collecting tank, milkers hand and bucket) using disposable gloves in to sterile plastic bags.

Milk samples were collected after the teats were scrubbed vigorously with a pledge of cotton moistened with 70% ethyl alcohol and the first 3-4 streams of milk were discarded. The nearest teats were sampled first, then toward far ones. The collecting vial was held as near horizontal as possible and by turning the teat to a near horizontal position.Approximately 10 ml of milk from four teats were collected in a sterile universal bottle after the cows were restrained in self-locking stanchions. Swabs from abattoir (carcass and Pooled butchers hand swab, knife swab and pooled hanging material) and swabs from dairy farms before the beginning of milking process (Pooled milkers' hand swab, tank swab and pooled buckets swab) were collected by using a sterile cotton swab in buffered peptone water (BPW). The faecal specimens of abattoir slaughtered beef were from the caecum and farm lactating cows were directly taken from the rectum and collected in a clean sterile air tight stool cup. The samples were transported using an ice box and analyzed at microbiology laboratory of Addis Ababa University College of veterinary medicine and agriculture.

Isolation and Identification of *Salmonellae*: The isolation and identification of *Salmonella* was performed at the microbiology laboratory of College of veterinary medicine and agriculture using techniques recommended by International Organizations for Standardization [22], The isolation and identification involved; 2 gm of faecal sample and 5 ml of milk werepre-enriched with 18 ml and 45 ml of buffered peptone water (BPW) respectivelyand then samples enriched with BPW were incubated for 24 hrs at 37°C (Oxoid CM509, Basingstoke, England). A portion (0.1 ml) of the pre-enriched culture was transferred to 10 ml of selenite cysteine (SC) (Himedia M025, Mumbi) broth and another 0.1 ml portion was transferred to10 ml of Rappaport and Vassiliadis (RV) broth (Merck, Darmstadt, Germany) broth and incubated at 37°C and 42°C for 24 hours respectively. Finally, from the selective enrichment media the sample was streakedonto Salmonella Shigella agar (SSA)and Xylose Lysine Deoxycholate agar (XLD) (Oxoid CM0469, Basingstoke, England). The sub cultured plates were incubated at 37°C for 24 hrs and the incubation was prolonged to 48 hrs for those that did not show any growth during the 24 hrs incubation. The cultured plates, SSA and XLD agar were examined for the presence of typical colonies of *Salmonella* based on cultural and morphological characteristics, that is, transparent colonies with black centre on SSA and a slightly transparent zone of reddish color and a black center on XLD. The isolates were sub cultured on nutrient agar for isolation of pure culture and subsequent biochemical characterization.

All suspected non-lactose fermenting Salmonella colonies were picked from the nutrient agar and inoculated into Triple Sugar Iron (TSI), Urease broth and IMViC (Indole, Methyl red, VogesProskauer and Citrate) broths for biochemical conformation. Colonies that produced alkaline slant 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), negative for Voges-Proskauer, positive for citrate utilization, M-R positive and V-P negativewere considered to be Salmonella-positive [23].

Antimicrobial Susceptibility Test of Salmonella Isolates: Antimicrobial susceptibility and drug resistance pattern of *Salmonella* isolates was checked against 10common antibiotics from Oxoid, including, amoxicillin-clavulanic acid (AMC) 30 µg, Sulphamethoxazole trimethoprim (SXT) 25 µg, ciprofloxacin (CIP) 10 µg, chloramphenicol (C) 10 µg, kanamycin (KA) 30 µg, gentamycin (CN) 10 µg, nalidixic acid (NA) 30 µg, streptomycin (S) 10 µg, Cefoxitin (FOX) 30 µg and ampicillin (AMP) 10 µg were applied using Kirby- Bauer antibiotic discs diffusion method [24]. A suspension of *Salmonella* culture was made at 0.5 McFarland turbidity standard and spread with a sterile cotton swab over the entire surface of Mueller Hinton agar (Oxoid) plates. After the inoculum was dried for about 5 minutes, the standard antibiotic disks each containing a specific concentration of antibiotics was

Table 1: Zone of inhibition and standard composition of antibiotic for susceptibility testing

Measurement of zone of inhibition (mm)				
Antimicrobials	Potencyof disc	Susceptible ≥	Intermediate	Resistant ≤
Amoxicillin	25µg	18	14-17	13
Ampicillin	10µg	17	14-16	13
Gentamycin	10µg	15	13-14	12
Chloramphenicol	5µg	31	21-30	20
Cefoxitin	30µg	18	13-17	12
Ciprofloxacin	30µg	18	15-17	14
Kanamycin	30µg	18	14-17	13
Streptomycin	10µg	15	12-14	11
Nalixidic acid	30µg	19	14-18	13
Sulphamethoxazole trimethoprim	25µg	16	11-15	10

applied per plate. The plates were inverted and incubated at 37°C for 18 to 24 hours. After incubation, the diameters of the inhibition zones were measured in millimeters and interpreted in accordance with Clinical Laboratory Institute Standards [24].

Data Analysis: Data was analyzed using SPSS version 13 computer software (SPSS 13.0 Command Syntax Reference. SPSS Inc., Chicago, 2004) and presented in tables and graphs. The Chi-square test was utilized to assess significant differences in antimicrobial resistance of *Salmonella* isolates from abattoir and farm; from isolates of carcass and faeces of beef and from isolates of milk and faeces of cows. A difference was taken as significant at a p-value less than 0.05.

Ethical Consideration: The study was ethically approved by the Addis Ababa University College of veterinary medicine and agriculture. More over both informed and written consent were obtained from the human subjects.

RESULTS

Distribution of *Salmonella* in Abattoir and Dairy Farms of Addis Ababa City: From a total of 201 samples collected from the abattoir and dairy farm tested, 15(7.5%) were positive for Salmonella (Table 2). Out of 108 samples tested from the abattoir, the overall percentage prevalence of Salmonella was 11(10.2%) with prevalence rates of 10, 10, 33.3, 0 and 16.7%, for carcass swab, feces, pooled hanging material, pooled butchery hand and pooled knife of the slaughter house, respectively. Out of 93 samples tested from the dairy farms, the overall percentage of salmonellae was 4(4.3%) with prevalence rates of 0, 9.4, 0, 0, 0 and 14% for udder milk, feces, tank milk, bucket swab, hand swab and tank swab respectively.

Table 2: Salmonella isolates from abattoir and dairy farms

	Number of samples		
Source of samples	Examined	Positive	Percentage
Abattoir (total)	108	11	10.2
Carcass swab	60	6	10
Feces	30	2	6.7
Pooled carcass hanging material swab	6	2	33.3
Pooled butchery hand swab	6	0	0
Pooled abattoir knife swab	6	1	16.7
Dairy farm (total)	93	4	4.3
Udder milk	33	0	0
Cow feces	32	3	9.4
Tank milk	7	0	0
Tank swab	7	1	14.3
Pooled bucket swab	7	0	0
Pooled milkers hand swab	7	0	0
Overall total sample	201	15	7.5

Antimicrobial Susceptibility Test on *Salmonella* Isolates from Abattoir and Dairy Farms of Addis Ababa: The antimicrobial susceptibility pattern of the 15isolates indicated that 86.9, 73.3, 67.7, 60 and 53.3% were resistant to ampicillin, Kanamycin, Nalidixic acid, amoxicillin and cefoxitin, respectively. On the other hand the 15 isolates were, 100, 93.3, 73, 60 and 53.3 sensitive to ciprofloxacillin, gentamycin, streptomycin, Sulphamethoxazole-Trimethoprimandchloramphenicol, respectively.

Multi-Drug Resistance Pattern of the 15 Isolates Derived from Addis Ababa City: From the 15 isolates of *Salmonella*, 13 (86.7%) of both abattoir and dairy farm isolates showed resistance for two or more of the antimicrobials tested. From these resistance isolates, most of them (23%) showed resistance to Ampicillin, chloramphenicol, kanamycin, nalidixic acid, sulphamethoxazole-trimethoprim,cefoxitinandamoxicillin-clavulanic acid followed by resistance to ampicillin, kanamycin and nalidixic acid (15.4%). It was also evident from the result that 63.6% of abattoir isolates showed multiple antimicrobial resistances to 50% of the antimicrobials tested (Table 4).

Table 3: Antibiotic Resistance Profile of *Salmonella* isolates

Antibiotics	Conc. (µg)	Number of susceptible	Intermediates	Numberresistant
Ampicillin	10	2(13.3%)	0(0%)	13(86.9%)
Chloramphenicol	30	8(53.3%)	0(0%)	7(46.7%)
Ciprofloxacin	10	15(100%)	0(0%)	0(0%)
Streptomycin	10	11(73.3%)	4(26.7%)	0(0%)
Kanamycin	30	4(26.7%)	0(0%)	11(73.3%)
Gentamycin	10	14(93%)	0(0%)	1(6.7%)
Nalidixic acid	30	5(33.3%)	0(0%)	10(67.7%)
Sulfamethoxazole-Trimethoprim	25	9(60%)	0(0%)	6(40%)
Cefoxitin	10	746.7%)	0(0%)	8(53%)
Amoxicillin	30	5(33.3%)	1(6.7%)	9(60%)

Table 4: Multiple antimicrobial resistance profile of *Salmonella* isolates from abattoir and dairy farms

Antibiotics	Number of drugs	Number of isolates
AMP, C, KA, CN, NA, SXT, FOX, AMC	8	1
AMP, C, KA, NA, SXT, FOX, AMC	7	3
AMP, KA, NA, SXT, AMC	5	1
AMP, C, KA, NA, AMC	5	1
AMP, C, KA, NA, FOX	5	1
AMP, C, KA, SXT, AMC	5	1
AMP, KA, NA	3	2
KA, NA, ALC	3	1
AMP, FOX, AMC	3	1
AMP, FOX	2	1
Total		13

Table 5: Multi-drug susceptibility pattern of *Salmonella* isolates from abattoir and dairy farmsfor each isolated sample

	Antibiotic sensitivity of *Salmonella* isolates									
	Antibiotics									
Isolates number	AMP	C	CIP	S	KA	CN	NA	SXT	FOX	AMC
FF3	R	S	S	S	S	S	S	S	R	R
FF2	R	S	S	S	S	S	S	S	R	S
TSF4	R	S	S	I	R	S	R	R	S	R
FF5	S	S	S	S	S	S	S	S	R	S
ACS3	R	R	S	S	R	R	R	R	R	R
ACS6	R	S	S	S	R	S	R	S	S	I
ACS23	S	S	S	S	R	S	R	S	S	R
ACS39	R	S	S	I	S	S	S	S	S	S
ACS43	R	R	S	I	R	S	R	R	R	R
ACS54	R	S	S	S	R	S	R	S	S	S
AF7	R	R	S	S	R	S	R	R	R	R
AF18	R	R	S	I	R	S	R	S	R	S
PSS3	R	R	S	S	R	S	S	R	S	R
PSS6	R	R	S	S	R	S	R	R	R	R
PKS5	R	R	S	S	R	S	R	S	S	R

AMC: Amoxicillin-clavulanic acid, C:Chloramphenicol, CIP: Ciprofloxacin, S:Streptomycin, KA: Kanamycin, CN: Gentamycin, NA: Nalidixic acid, SXT: Sulphamethoxazole-trimethoprim, FOX: Cefoxitin, AMP:Ampicillin.

FF: fecal sample from lactating dairy farm, TSF: tank swab of the farm, ACS: abattoir carcass swab, AF: fecal sample from abattoir, PSS: pooled carcass hanging material swab and PKS: pooled carcass knifes swab

Salmonella isolated from abattoir and dairy farms samples showed 86.7% resistance to ampicillin and followed by Kanamycin and Nalidixic acid which were 73.3 and 66.7%, respectively. Whereas, all of the isolates were 100% sensitive to ciprofloxacin and 93.3% to gentamycin. All abattoir faecal isolates of beef showed 100% resistance to ampicillin, chloramphenicol, Kanamycin, Nalidixic acid and cefoxitin. All the pooled swab isolates

of the abattoir showed 100% resistance for ampicillin, chloramphenicol, Kanamycin and amoxicillin-clavulanic acid (Table 5).

DISCUSSION

Salmonella is an important zoonotic pathogen and its prevalence in animals poses a continuous threat to man [8]. In this study, 15(7.5%) *Salmonella* were isolated from abattoir 11(10.8%) and dairy farm 4(4.3%). There was no significant association in the prevalence of Salmonella among the different sample sources analyzed (p = 0.246). From the outcome of this research undertaken it was evident that there is high prevalence rates of salmonella isolated from abattoir compared to the isolates from dairy farms. This is consistent with various reports made in the country and elsewhere in the world [5, 13, 25-27].

Distribution of *Salmonella* in Abattoir and Selected Dairy Farms of Addis Ababa City: In this study the overall prevalence of *Salmonella* in abattoir of Addis Ababa was (10.76%) with prevalent rates of 13.3, 33.3 and 16.7% from apparently slaughtered cattle, pooled carcass hanging material and pooled carcass splitting knifes, respectively. The overall result is higher(10.76%) as compared to other studies [15, 25, 26]. The reason could be associated with the hygienic status of the abattoir and cross contamination among the materials used in the slaughtering operation and processing of food. Alemayehu *et al.* [25] reported a prevalence of 7.1% from apparently healthy slaughtered cattle which are less than the present report. This difference may be attributed to the difference in the tests used, since pre-enrichment steps using buffered peptone water was employed in this study; results of the present findings could be attributed to contamination of the red meat at abattoir at any stage during butchering and feces of slaughtered animals, which are asymptomatic carrier, this is similar with the study of Zewdu and Cornelius [15] and Wray and Davies [28]. In this study Salmonella prevalence in apparently healthy lactating dairy cows and milk in contact materials was low. Only (4.3%) (4 of 93) of samples gave positive results. Addis *et al.* [29] reported a prevalence of 10.76 from apparently health lactating dairy cattle which are much higher than the present report. The low level of detection could probably be due to the low prevalence of this pathogen in lactating animals and improved hygienic status of the farms.

On the other hand reports from England (0.2 and 4%) and from Northern Thailand (3%) are lower than the severalinvestigations [30-32] but a report from Cameroon by Akoachere *et al.* [33] indicated a very high prevalence (27%) of Salmonella among cattle. This may be due to the difference in the living condition, like housing conditions, feeding habits, types of feed given for the cattle, of the two cattle populations.

Antimicrobial Susceptibility Test on *Salmonella* Isolates from Abattoir and Selected Dairy Farms of Addis Ababa City: The antimicrobial susceptibility patterns of the *Salmonella* isolates indicated that a large proportion of the isolates were resistant to a variety of the drugs tested particularly ampicillin, Kanamycin, nalidixic acid, amoxicillin and cefoxitinwith resistance rate of 86.9, 73.3, 67.7, 60 and 53.3% respectively.The percentages of resistance obtained with these antibiotics are comparable with those reported in other studies in Ethiopia [34].

All the isolated *Salmonella*, in the current study, were 86.9% resistant to ampicillin. This finding is comparable with previous reports by Suresh *et al.* [35] from Nigeria, Akinyemia *et al.* [36] from Cameroonand Akoachere *et al.* [33], whoreported a comparable 90, over 90 and 100% resistance to ampicillin, respectively. Hghi *et al.* [37] reported a resistance rate of 60.3 and72.7% in different study periods among human isolates from Iran, which is slightly lower than the current finding.

In the present study, all *Salmonella* isolates were susceptible to Ciprofloxacin and only one was resistant togentamycin. Thatmightbe explained by the limited availability and high cost of the above groups of antimicrobials that would reduce their frequent utilization in veterinary practice or public health practices in Ethiopia.

Multi-Drug Resistance Pattern of the 15 Isolates Derived from Addis Ababa City: Resistance for two or more of antimicrobials (86.7%) which was observed in this study ishigher than most studies conducted in Ethiopia [30-32] and elsewhere in the world [7, 8]. This difference may be due to the increasing rate of inappropriate utilization of antibiotics in the dairy farms which favors selection pressure that increased the advantage of maintaining resistance genes in bacteria [38, 39]. Addis *et al*. [29] (2011) reported a comparable result of 83.3%.

In Ethiopia, Alemayehu *et al.* [25] showed 52% of the salmonellae isolated at the slaughter house from beef were resistant to atleast three antibiotics. Addis *et al.* [29] reported that the isolates of *Salmonella* from apparently health lactating dairy cattle and personnel from Addis Ababa were resistant to the commonly used antibiotics including ampicillin, streptomycin,

nitrofurantoin, kanamycin and tetracycline. The result of the current research also indicated resistance of Salmonella isolates to commonly used antimicrobials

CONCLUSIONS

Higher proportion of *Salmonellae* was isolated from abattoir than dairy farms. High proportion of *Salmonella* isolates developed resistance to commonly prescribed antimicrobials. So, the current study indicated that, wise use of antimicrobials must be practiced to combat the ever increasing situation of antimicrobial resistance and the necessity of a further investigation on the prevalence and antimicrobial susceptibility pattern of *Salmonella*.

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