

ADDIS ABABA UNIVERSITY COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE



**ISOLATION, IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE
OF SALMONELLA ISOLATES FROM DAIRY FARMS IN AND AROUND BATU,
OROMIA, ETHIOPIA.**

BY:

ABDIAZIZ ABDULAH

ADVISOR:

Dr. Fufa. Abunna (Associate Professor)

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Isolation, Identification and Antimicrobial Susceptibility Profile of *Salmonella* isolates from Dairy Farms in and Around Batu, Oromia, Ethiopia.

By: Abdiaziz Abdulahi

Board of external examiners

Chairperson

signature

Dr. Yacob Hailu

CVMA, AAU

External examiners

1. Dr. Kebede Amenu

Hawassa University

2. Dr. Aster Yohannes

Academic advisor:

Dr. Fufa Abunna

June, 2016

Bishoftu, Ethiopia

TABLE OF CONTENTS	PAGES
CONTENTS.....	i
ACKNOWLEDGEMENTS	ii
LIST OF TABLES.....	iii
LIST OF ANNEXES.....	iv
LIST OF ABBREVIATIONS.....	v
ABSTRACT.....	vi
1.INTRODUCTION	1
2. MATERIALS AND METHODS	4
2.1. Study Area	4
2.2. Study Population.....	4
2.3. Study Design	4
2.4. Sampling Methods and Sample Collection	5
2.5. Isolation and Identification of <i>Salmonella</i>	5
2.5.1. Pre-Enrichment in Non-Selective Liquid Medium	6
2.5.2. Enrichment in Selective Liquid Media.....	6
2.5.3. Plating Out and Identification	6
2.5.4. Biochemical Confirmation of <i>Salmonella</i> Isolates.....	7
2.6. Antimicrobial Susceptibility Testing.....	7
2.7. Data Management and Analysis.....	8
3. RESULTS.....	9
3.1. Frequency of Isolation of <i>Salmonella</i>.....	9
3.2. Antimicrobial Resistance of <i>Salmonella</i>.....	11
4. DISCUSSION.....	13
5. CONCLUSION AND RECOMMENDATIONS.....	16
6. REFERENCES.....	17
7. ANNEXES.....	Error! Bookmark not defined.

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LIST OF TABLES

PAGES

Table 1: Distribution of *Salmonella* Isolates among Dairy Farms by Sample Types.....10
Table 2: Antimicrobial susceptibility test results of *salmonella* isolates from dairy farms.....11
Table 3: Multiple Antimicrobial Resistance Patterns of *Salmonella* Isolates from Dairy Farms.12

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LIST OF ANNEXES

PAGES

Annex 1: Type and Preparation of Microbiological Media Used For Isolation, Identification and Antimicrobial Susceptibility Testing Of *Salmonella*.....20

Annex 2: Performance Standards for Antimicrobial Susceptibility Testing Of *Salmonella*.....24

Annex 3: Plating and Biochemical Tests Record Sheet Format Used For *Salmonella* Isolation.....25

Annex 4: Flow diagram showing ISO method for detection of *Salmonella*26

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LIST OF ABBREVIATIONS

BGA	Brilliant-Green Agar
CI	Confidence Interval
CLSI	Clinical and Laboratory Standards Institute
ISO	International Organization for Standardization
MKTTn	Muller-Kauffman Tetrathionate with Novobiocine
MR-VP	Methyl Red- Voges-Proskauer
NA	Nutrient agar
NCCLS	National Committee on Clinical Laboratory Standards
RV	Rappaport-vasillidias
SPSS	Statistical Product and Service Solution
TSI	Triple sugar iron agar
TT	Tetrathionate
XLD	Xylose lysine deoxycholate

ABSTRACT

Antimicrobial-resistant *Salmonella* and other zoonotic bacterial pathogens can be transferred from animals to humans through consumption of contaminated food and food products and thus present a public health risk. The increase in *Salmonella* resistance to the commonly used antimicrobials both in the public health and veterinary sectors is one of the major threats of health care worldwide. The objectives of this study were to isolate and identify *Salmonella* spp from 225 samples obtained from lactating dairy cows, personnel and equipment's at farms, Batu, Ethiopia; and to determine the *in-vitro* antimicrobials susceptibility profile of the isolates. Cross-sectional study was conducted on apparently healthy lactating dairy cows, personnel and equipment's at dairy farms in Batu, Ethiopia from January 2016 to May 2016. The samples were examined for the presence of *Salmonella* spp following standard techniques and procedures outlined by the international organization for standardization. *Salmonella* was isolated from 11.1% of the total samples. *Salmonella* organisms were isolated in the studied dairy farms; the isolation rate varies from 0.0% to 27.3% among the dairy farms. The number of *Salmonella* isolates among the dairy farms by sample types were bucket milk 2(25.0%), bucket swab 1(9.1%), feces 2(10.5%), hand swab 3(20.0%), tank milk 0(0.0%), tank swab 2(14.3%), udder milk 15(11.3%). Out of the 25 isolates subjected to antimicrobial susceptibility testing, accordingly, 96% (24/25), 92% (23/25), 84% (21/25) and 80% (20/25) of isolates showed resistance for Ampicillin, Amoxicillin, Cefoxitin and Chloramphenicol respectively Ciprofloxacin was the best drug followed by Gentamicin. Out of the 25 isolates 24 were resistant to antimicrobials tested. Among the 24 resistant isolates, 100% (24/24) showed multiple antimicrobials resistance (resistance to four or more antimicrobials). Results of the present study indicated that dairy farms are potential sources of multiple antimicrobials resistant *Salmonella*. Therefore, further studies should be carried out on antimicrobial resistance pattern of *Salmonella*, by taking in to consideration that *Salmonella* is a potential food borne pathogen.

Key words: Antimicrobial, Batu, Isolate, Milk, Resistant, salmonella

1. INTRODUCTION

Salmonella is cited as in the most common causative agent of food borne illness (Cardinal *et al.*, 2005), The United States alone reported an estimated 1.4 million total cases of non-typhoidal *Salmonella* per year (Garry *et al.*, 2009). The large number of outbreaks in developed and developing countries produced by this bacteria indicates its importance and impact (Bell and Kyriakides, 2002). Salmonellosis is not only responsible of a large number of illnesses but also there is a cost associated with these outbreaks which in United States has been estimated to range from \$600 million to \$3.5 billion each year (Ter-Hsinet *et al.*, 2005).

Some *Salmonella* species are host adapted. *S. typhi* and *S. paratyphi* cause typhoid and paratyphoid fever in humans; *S. pullorum* and *S. gallinarum* are animal host-adapted *Salmonella* species in chicken and turkey. Some strains mainly produce infection in animals but could also affect human, e.g. *S. Dublin* in cattle, *S. Cholerae* in swine (Bell and Kyriade, 2002; Molbaketal, 2006).

Food borne illnesses are often time caused by non-typhi *Salmonella* species. This group includes over 2,500 serotypes that are found in the gastrointestinal tracts of birds, mammals, reptiles and insects (Molbaketal, 2006). Meats and eggs has been considered for long time to be the principal vectors for transmission, but cheddar cheese, ice cream, milk and milk powders, pasta, peanut butter, chocolate, and more recently cantaloupes, tomatoes, alfalfa sprouts, spices have caused Salmonellosis as well (Bell and Kyriade, 2002; Molbaketal., 2006). As the food chain becomes more integrated and the food chain expands further many other food items will be involved in cases of Salmonellosis (Bell and Kyriakides, 2002).

Salmonella belongs to the family Enterobacteriaceae (Guthrie, 1991). The genus *Salmonella* contains two species; *S. enterica* and *S. bongori*, which was formerly subspecies V. Six subspecies are differentiated within *S. enteric* a based on their biochemical and genomic characteristics, a Roman

numeral and a name are used for the designation of these six subspecies as follows: I, *S. enterica* subsp. *enterica*; II, *S. enterica* subsp. *salamae*; IIIa, *S. enterica* subsp. *arizonae*; IIIb, *S. enterica* subsp. *diarizonae*; IV, *S. enterica* subsp. *houtenae*, and VI, *S. enterica* subsp. *indica* (Brenner *et al.*, 2000).

With regard to food safety, *S. enteric* subsp. *enteric* is the subspecies of most concern because the strains within these sero groups are known to cause 99% of *Salmonella* infections in humans (Bell and Kyriakides, 2002; Brenner *et al.*, 2000).

Salmonella are facultative anaerobic, gram negative, small rods, motile (Bell and Kyriakides, 2002; Molbak *et al.*, 2006). Temperature for growth ranges from 8°C to 45°C, strains can stand pH between 4 to 9, and is able to grow at water activities above 0.94. *Salmonella* is heat labile so the organism can be inactivated at ordinary cooking temperatures (> 70 °C) although the cooling time and values for temperature and time could change depending on the serotype and the food matrix. In addition *Salmonella* has been shown to tolerate up to 20% salt concentration (Bell and Kyriakides, 2002; Guthrie, 1991). Under freezing conditions (from -23°C to -18°C) this microorganism is able to survive as long as seven years (Bell and Kyriakides, 2002).

The difficulty in controlling *Salmonella* is due to its ability to survive extreme environmental conditions (Guthrie, 1991).

Infections caused by *Salmonella* serotypes can produce enteric fever, gastroenteritis, and bacteremia or septicemia conditions (Guthrie, 1991; Monteville and Mathews, 2008). *Salmonella* Typhi and Paratyphi are responsible for causing enteric fever (Guthrie, 1991). The period of incubation for this infection ranges from 8 to 28 days and the common symptoms include fever, diarrhea, abdominal pain, headache (Monteville and Mathews, 2008). The antibiotics of choice for treatment of enteric fever are Chloramphenicol, Ampicillin or Trimethoprim-Sulfamethoxazole (Monteville and Mathews, 2008). When the infection is due to the consumption of a food item contaminated with non-typhoid *Salmonella* strains, the disease is often self-limiting in healthy individuals. Symptoms appear 8 to 72 hours after ingestion, and are less severe than in the previous case, and non-bloody diarrhea

and abdominal pain disappear within 5 days. The treatment is based more on fluid and electrolyte replacement than on antibiotic use. Infections caused by nontyphoid *Salmonella* serotypes can also evolve into systematic infections followed by chronic conditions (Monteville and Mathews, 2008). Salmonellosis occurs when the bacteria have been able to survive the low pH in the stomach and reach the mucosa in the small intestine in adequate numbers to cause infection. Epithelial cells localized in the mucosa mid layer are responsible to cover completely the *Salmonella* cells, which drive an inflammatory response (Guthrie, 1991). The infection could progress to acute levels, depending on the serotype causing the illness (Guthrie, 1991).

Despite the presence of a number of published works on different food items, limited information is still available therefore, the purpose of the present study was:-

- ✓ To isolate and identify *Salmonella* from different dairy farms in and around Batu and
- ✓ To determine the antimicrobials susceptibility profile of the isolates

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2. MATERIALS AND METHODS

2.1. Study Area

The present study was conducted from January 2016 to May 2016 at Batu; a town and separate districts in central Ethiopia. Batu in Oromia Region (region) with its 49,416 residents is a town located in Ethiopia - about 76 mi (or 122 km) south of Addis Ababa, the country's capital place.

Batu has a latitude and longitude of 7°56'N 38°43'E with an elevation of 1643 meters above sea level. Adjacent to Lake Batu (Lake Dambal), the economy of the town is based on fishing and horticulture. Batu is also home to a prison and a caustic soda factory.

2.2. Study Population

The study population or animals were apparently healthy dairy cows found in different dairy farms in and around Batu, which are mostly backyard or small scale dairy farms.

2.3. Study Design

A cross-sectional study ([observational study](#)) that involves the analysis of data collected from a population, or a representative subset, at one specific point in time— was conducted on apparently healthy dairy cows at Batu town from January 2016 to May 2016, to isolate and identify *Salmonella* by conventional cultural and biochemical methods and to determine the *in-vitro* antimicrobials susceptibility profile of isolates to a panel of nine antimicrobial agents.

The sampling days were randomly assigned to each dairy farm. During the study period, each dairy farm was visited once. Prior to the sample collection, cooperation letter was sent to dairy farms. All farm owners and milkers were volunteers and cooperating people really.

2.4. Sampling Methods and Sample Collection

Sampling method done was simple random sampling method for isolation, identification and drug susceptibility of *Salmonella* from dairy cows in and around Batu, Ethiopia. Samples collected were udder milk, tank milk, bucket milk, tank swab, bucket swab, feces, and milkers hand swab.

Samples were transported to the laboratory after being collected in a portable cooler container with ice packs (at 4 °C) and microbiological analysis was carried out immediately.

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2.5. Isolation and Identification of *Salmonella*

There are four steps for the recovery of injured *Salmonella* cells from a food matrix. First the pre-enrichment, where buffered peptone water or lactose broth can be used, This is followed by enrichment in selective broth, such as Rappaport- Vasilliadis (RV) broth, Selenite Cysteine Broth (SC), or tetrathionate broth (TT) and the subsequent isolation is done on selective Brilliant green agar, Salmonella-shigella agar, or XLD. Finally biochemical confirmation of salmonella isolates by using different biochemical tests that included TSI agar, Simon's citrate agar, urease, indole and MR-VP tests. (Molbak *et al.*, 2006). *Salmonella* was isolated and identified according to the techniques recommended by the International organization for standardization (ISO-6579, 2002) and Quinn *et al.*, (2004). The bacteriological media were prepared according to manufacturer's recommendations (Annex 1.).

2.5.1. Pre-enrichment in non-selective liquid medium

Up on arrival, all samples were processed separately. The samples were pre-enriched in appropriate amount of buffered peptone water in (1: 9) ratio and incubated at 37°C for 24 hrs.

2.5.2. Enrichment in selective liquid media

Rappaport- Vassiliadis medium (RV) broth and Müller Kauffman Tetrathionate with novobiocin (MKTTn) broth were used for selective enrichment of the samples. About 0.1 ml of the pre-enriched sample was transferred into a tube containing 10 ml of Rappaport- Vassiliadis medium (RV broth) and incubated at 42 °C for 24 hours. Another 1ml of the pre-enriched broth was transferred into a tube containing 10ml of MKTTn broth and incubated at 37°C for 24 hours.

2.5.3. Plating out and identification

Xylose lysine deoxycholate (XLD) agar and brilliant green agar (BGA) plates were used for plating out and identification. A loop full of inoculums from each RV and MKTTn broth cultures were plated onto XLD and BGA plates and incubated at 37 °C for 24 hours. After incubation, the plates were examined for the presence of typical and suspect colonies. Typical colonies of *Salmonella* grown on XLD-agar have a black center and a lightly transparent zone of reddish color due to the color change of the media (ISO 6579,2002) while H₂S negative variants grown on XLD agar are pink with a darker pink center. Lactose-positive *Salmonella* grown on XLD agar are yellow with or without blackening. Typical colonies of *Salmonella* on BGA are pink, 1 mm to 2 mm in diameter, and cause the color of medium to change to red. Typical or suspected colonies were selected from the selective plating media, streaked onto the surface of pre-dried nutrient agar plates and incubated at 37°C for 24hrs.

2.5.4. Biochemical confirmation of *Salmonella* isolates

Biochemical tests were done according to (ISO-6579, 2002) by using different biochemical tests that included TSI agar, Simon's citrate agar, urease, indole and MR-VP tests.

Isolate presumptive of salmonella for all biochemical tests were cultured on nutrient agar (NA) (HIMEDIA) for antimicrobial susceptibility testing.

2.6. Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing of the isolates was performed by using the disc diffusion method according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 2002) and (CLSI, 2013). Well-isolated colonies from nutrient agar plates were transferred into tubes containing 5 ml saline water. The culture was incubated at 37°C for 4 hours until it achieved the 0.5 McFarland turbidity standards. Sterile cotton swab was dipped into the suspension, rotated several times, pressing firmly on the inside wall of the tube above the fluid level to remove excess inoculum and swabbed uniformly over the surface of Muller Hinton agar plate (Oxoid, England). The plates were held at room temperature for 30 min to allow drying. The susceptibilities of the isolates were tested for the following antibiotic discs: Ampicillin (AMP) 10 µg, Amoxicillin (AMX) (30 µg), Gentamicin (CN) 10 µg, Kanamycin (K) 30 µg, Ciprofloxacin (CIP) 5 µg, Cefoxitin (FOX) 30µg, Chloramphenicol (C) 30 µg, Nalidixic Acid (NA) 30 µg and Streptomycin (S) 10 µg, were placed at least 15 mm apart and from the edge of the plates to prevent overlapping of the inhibition zones. The plates were incubated at 37°C for 24 h. The diameters of clear zones produced by antimicrobial inhibition of bacterial growth were measured to the nearest mm using a transparent straight line ruler and classified as resistant, intermediate, or susceptible according to the interpretive standards of the Clinical Laboratory Standards Institute (CLSI, 2013) (Annex 2.).

2.7. Data Management and Analysis

The data generated from the study were entered into Excel spread sheet (Microsoft®office excel 2013) and prepared for analysis. Descriptive statistics were performed using SPSS version. 20 statistical and also Stata 11. Descriptive was used to describe the result of proportion analysis. Proportion was estimated as the number of samples detected positive to salmonella isolation from the total sample analyzed. Chi-square and fisher's exact tests were done to study association between salmonella isolates and risk factors (sample type, farm type) using IBM SPSS 20. The significance level was set at 0.05.

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3. RESULTS

3.1. Frequency of Isolation of *Salmonella*

Of the 225 udder milk, bucket milk, tank milk, milkers hand swab, bucket swab, tank swab and fecal samples examined, 25 (11.1%) *Salmonella* isolates were obtained. There was no statistically significant difference in *Salmonella* isolation frequency between dairy farms ($X^2=9.134$; $df=9$; p -value=0.425).

Salmonella organisms were isolated in the studied dairy farms; the isolation rate varies from 0.0% to 27.3% among the farms. The number of *salmonella* isolates among dairy farms by sample types were bucket milk 2(25.0%), bucket swab 1(9.1%), feces 2(10.5%), hand swab 3(20.0%), tank milk 0(0.0%), tank swab 2(14.3%), and udder milk 15 (11.3%) (Table 1). There was no statistically significant difference in the isolation of *Salmonella* between sample types ($P= 0.414$).

Out of the dairy cows sampled, 11.9% (17/152) were positive for *Salmonella*, either from milk or feces. Of these cows, 88.2% (15/17) were positive from udder milk and 11.8% (2/17) was positive from fecal samples. None of the cows were positive for *Salmonella* from both feces and milk sample.

Table 1. Distribution of *Salmonella* Isolates among Dairy Farms by Sample Types

Origin	Type Of Samples	Number Of Samples Examined	Positives (%)
Dairy farms	Bucket Milk	8	2(25.0)
	Bucket Swab	11	1(9.1)
	Feces	19	2(10.5)
	Hand swab	15	3(20.0)
	Tank milk	25	0(0.0)
	Tank swab	14	2(14.3)
	Udder milk	133	15(11.3)
Total		225	25(11.1%)

3.2. Antimicrobial Resistance of *Salmonella* isolates

Out of the 25 *Salmonella* isolates, 24 of them were resistant to antimicrobial agents tested. Accordingly, 96% (24/25), 92% (23/25), 84% (21/25) and 80% (20/25) of isolates showed resistance for ampicillin, amoxicillin, cefoxitin and chloramphenicol respectively (Table 2). On the other hand, ciprofloxacin was the best, unfortunately I used ciprofloxacin to few samples since I couldn't find this drug at that time. Isolates tested against ciprofloxacin showed a great zone of inhibition. Among the 24 resistant isolates, 100% (24/24) showed multiple antimicrobials resistance (resistance to four or more antimicrobials) (Table 3).

Table 2. Antimicrobial Susceptibility Test Results of *Salmonella* Isolates from Dairy Farms

Antimicrobial agents	No. of isolates tested	No. of isolates (%)		
		susceptible	intermediate	resistant
Amoxicillin	25	0(0.00)	2(8.00)	23(92.00)
Ampicillin	25	0(0.00)	1(4.00)	24(96.00)
Cefoxitin	25	1(4.00)	3(12.00)	21(84.00)
Chloramphenicol	25	2(8.00)	3(12.00)	20(80.00)
Gentamycin	20	5(25.00)	8(40.00)	7(35.00)
Kanamycin	24	0(0.00)	6(25.00)	18(75.00)
Nalidixic Acid	23	2(9.09)	3(13.63)	18(78.26)
Streptomycin	24	1(4.17)	4(16.7)	19(79.17)
Ciprofloxacin	3	3(100.00)	0(0.00)	0(0.00)

Table 3. Multiple Antimicrobial Resistance Patterns of *Salmonella* Isolates from Dairy Farms

Number Of Antimicrobials	Antimicrobial Resistance Pattern (No. Of Isolate)	No. Of Isolates resistant (%)
Four	AMX,AMP,FOX,S*(2)	5(20.83)
	AMX,AMP,C,S*(1)	
	AMX,AMP,C,K*(1)	
	AMP,C,NA,S*(1)	
Five	AMX,AMP,FOX,C,S*(1)	3(12.5)
	AMX,AMP,C,K,NA*(1)	
	AMX,AMP,FOX,C,CN*(1)	
Six	AMX,AMP,FOX,C,K,NA*(2)	3(12.5)
	AMX,AMP,FOX,K,NA,S*(1)	
Seven	AMX,AMP,FOX,C,K,NA,S*(7)	8(33.33)
	AMX,AMP,FOX,CN,K,NA,S*(1)	
Eight	AMX,AMP,FOX,C,CN,K,NA,S*(5)	5(20.83)

Abbreviations: AMX=amoxicillin, AMP=ampicillin, FOX=cefoxitin, C=chloramphenicol, CN=gentamycin, K=kanamycin, NA=nalidixic acid, S=streptomycin, CIP=ciprofloxacin.

4. DISCUSSION

In the present study, from the total of 225 different samples from dairy farms examined for *Salmonella*, 11.1% (25/225) were positive; of which *Salmonella* organisms were isolated of the studied dairy farms. The number of *Salmonella* isolates among dairy farms by sample types were bucket milk 2(25.0%), bucket swab 1(9.1%), feces 2(10.3%), hand swab 3(20.0%), tank milk 0(0.0%), tank swab 2(14.3%), and udder milk 15 (11.3%). This was in agreement with the study conducted in Addis Ababa. From the total of 195 dairy cows tested, 10.76% (21/195) were positive for *Salmonella*, in this study the prevalence of *Salmonella* in apparently healthy lactating dairy cows was (10.76%) (Zewdu and Cornelius, 2009).

But a report from Cameroon by (Akoachere et al., 2009) 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. On the other hand reports from England (0.2% and 4%) and from Northern Thailand (3%) are much lower than the current investigation (Davies *et al.*, (1999-2000) and Padungtod *et al.*, 2006). My study differs from study conducted in Jimma zone especially Kersa district 20% Prevalence and antimicrobial resistance of *Salmonella* isolated from samples in raw milk (Tadesse, 2012).

Out of 25 pooled buckets and tank swabs (11 bucket swab and 14 tank swab) examined, 9.1% of bucket swabs were positive to *Salmonella* and 14.3% of tank swabs were positive to *Salmonella*. Therefore both the bucket and the tank in to which the milk was collected can act as a source of milk contamination with *Salmonella*.

From 15 pooled hand swabs from dairy farms examined, 20.0% (3/15) samples were positive to *Salmonella*. Indicating that milkers' hands had a great role of *Salmonella* spread.

All the *Salmonella* isolates were tested against a panel of antimicrobials available at local market. Out of the 25 isolates 24 (96%) of them were resistant to one or more antimicrobials tested. Accordingly, higher resistance of 96%, 92%, and 84% was observed against Ampicillin, Amoxicillin and Cefoxitin respectively. These results were in agreement with the study conducted in Addis Ababa where all the isolates were 100% resistant to Ampicillin and also to commonly used antimicrobials including streptomycin and Kanamycin, Ciprofloxacin showed a good antimicrobial activity against both human and cow isolates the same to my result (Zewdu and Cornelius, 2009).

A total of twelve different antimicrobial resistance patterns were observed: five isolates resistant to eight antimicrobials, eight isolates was resistant to seven antimicrobials with two different resistant patterns, three isolates were resistant to six antimicrobials with two different resistant patterns, three isolates were resistant to five antimicrobials for three different resistance patterns and five isolates were resistant to four different antimicrobials with four different resistance patterns.

A total of 100 isolates were collected from poultry and swine sources from different points along the process and the environment. These isolates were tested for susceptibility to 6 antimicrobial agents of human health significance. Interesting, antibiotic resistance among poultry isolates was not observed although 88% of the isolates from poultry sources were two serotypes that have been reported to be resistant to at least two antibiotics tested in this study (tetracycline and Ampicillin) (Karczmarczyk *et al.*, 2010). Seventy two percent of the isolates from pork did not show resistance to any of the antimicrobials evaluated.

All *Salmonella* serotypes are considered pathogenic and therefore represent a hazard for public health. However, the rate of resistance to antibiotic agents varies among serotypes (Boyenet *al.*, 2008).

5. CONCLUSION AND RECOMMENDATIONS

In the present study, an overall of 11.1% *Salmonella* was isolated from 225 different types of samples examined from dairy farms. The isolation of this number showed that dairy cows and their environment are important sources of milk contamination with the organism, and consumption of raw milk and other unpasteurized dairy products can lead to infection with zoonotic Salmonellosis. The presence of high proportion of multiple antimicrobials resistant isolates (100%) in the dairy farms in this study further signifies the public health importance of *Salmonella*.

Therefore based on the present study and findings, the following points are forwarded:

- The present study indicated the need for good hygiene
- Rational use of antimicrobials in veterinary medicine
- Despite the problem of drug resistance still there are antimicrobials of choice to treat Salmonellosis so they must be used and informed the society like:- ciprofloxacin
- Public education of the risks coming from consuming raw milk or unpasteurized milks
- Further investigations and studies of *Salmonella* up to molecular characterization should be carried out.

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7. ANNEXES

Annex 1: Type and Preparation of Microbiological Media Used For Isolation, Identification and Antimicrobial Susceptibility Testing Of *Salmonella*

A) Buffered peptone water

Composition (g/Litre):

Enzymatic digest of casein 10.0 g

Sodium chloride 5.0 g

Disodium hydrogen phosphate dodecahydrate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) 9.0 g

Potassium dihydrogen phosphate (KH_2PO_4) 1.5 g

Water 1 000 ml

Preparation: Add 15 gram of the components in the 1000 ml of distilled water, Mix well And distribute into universal bottle of suitable capacity to obtain the portions necessary for

The test. Sterilize by autoclaving for 15 min in the autoclave set at 121 °C.

B) Rappaport -Vassiliadis (RV) enrichment broth of 500 g (Oxoid, England)

Composition (g/Litre):

- Soya peptone5.0 g

- Sodium chloride.....8.0 g

- Potassium dihydrogen phosphate1.6 g

- Magnesium chloride40.0 g

- Malachite green.....0.04 g

Preparation: Weigh 30 g (the equivalent weight of dehydrated medium per Litre) and add to 1 Litre of distilled water. Heat gently until completely dissolved. Dispense 10 ml volumes into screw capped bottles or tubes and sterilize by autoclaving at 115 °C for 15 minutes.

C) Muller-Kauffman Tetrathionate (Novobiocine enrichment broth) (Oxoid Ltd., Basingstoke Hampshire, England)

Composition (g/Litre):

Tryptone 7.0; Soya peptone 2.3; Sodium Chloride 2.3; calcium carbonate 25.0; Sodium thiosulphate 40.7 and ox bile 4.75

Preparation: Suspend 89.5g in one litre of demineralized water, heat briefly to boiling. Do not autoclave! After cooling, add 20ml iodine potassium-iodide solution. Dispense evenly any precipitate. Potassium iodine solution: (5g KI, 4g I, PH 8.0 \pm 0.2 at 25°C).

D) Xylose lysine deoxycholate agar (XLD agar) 500 g (Sifin, Berlin, Germany)

Composition (g/Litre):

Yeast extract.....	3.0
- L-Lysine hydrochloride.....	5.0
- Xylose.....	3.75
- Lactose.....	7.5
- Sucrose.....	7.5
- Sodium deoxycholate.....	1.0
- Sodium chloride.....	5.0
- Sodium thiosulphate.....	6.8
- Iron (III) ammonium citrate.....	0.8
- Phenol red.....	0.08
- Agar.....	16.5

Preparation □ Suspend 56.68gm in 1000 (1 Litre) of distilled water by heating, with frequent agitation, until the medium starts to boil. Avoid overheating. Adjust the pH, if necessary, so that after sterilization it is 7.4 at 25 °C. Heat with frequent agitation until the medium boils and the agar dissolves. Do not overheat. Transfer immediately to a water bath at 50 °C, agitate and pour into plates. Allow to solidify. Immediately before use, dry the agar plates carefully (preferably with the lids off and the agar surface downwards) in the oven set between 37 °C and 55 °C until the surface of the agar is dry. It is advisable not to prepare large volumes which will require prolonged heating.

E) BRILLIANT GREEN AGAR

Preparation: Suspend 29.0 g of the medium in one 500ml of distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure

(121°C) for 15 minutes. Avoid overheating

F) Nutrient agar

Composition (g/Litre):

- Lab-Lemco powder.....1.0
- Yeast extract2.0
- Peptone.....5.0
- Sodium chloride.....5.0
- Agar.....15.0

PH: 7.4 ± 0.2

Preparation: Dissolve 28g of the components or the dehydrated complete medium in 1000ml of distilled water, by heating if necessary. Sterilize for 15 min in the autoclave set at 121 °C. Transfer about 15 ml of the melted medium to sterile small Petri dishes and proceed.

G) Triple sugar/iron agar (TSI agar)

Composition (g/Litre): 'Lab-Lenco' powder 3.0; yeast extract 3.0; peptone 20.0; sodium chloride 5.0; lactose 10.0; sucrose 10.0; glucose 1.0; ferric citrate 0.3; sodium thiosulfate 0.3; Phenol red 9.5; agar 12.0. PH=7.4± 0.2 at 250C Preparation: Suspend 65 grams in one Litre of distilled water and bring to the boil to dissolve completely. Sterilize in the autoclave set at 121 °C for 15 minutes. Dispense the medium into test tubes or dishes in quantities of 10 ml Allow to set in a sloped form to give a butt of depth 2.5 cm to about 5 cm

H) Tryptophan Soya Broth for Indole test

Composition (g/Litre):

Casein enzymic hydrolysate 10.0

Sodium chloride 5.0

DL- Tryptophan 1.0

Preparation: Dissolve 30gm of the components of tryptone broth in one litre distilled water. Dispense 3 to 5 ml of the medium into each of tubes. Sterilize for 15 min in the autoclave set at 121 °C.

I) Muller – Hinton agar (Oxoid, England

Composition (g/Litre):

-Beef, dehydrated infusion from 300.0

-Casein hydrolysate 17.5

-Starch 1.5

-Agar 17.0

-pH 7.3 ± 0.1 at 25°C

Preparation: Add 38g to one litre of distilled water. Bring to the boil to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 minutes.

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Annex 2: Performance Standards for Antimicrobial Susceptibility Testing Of
Salmonella

Antimicrobials	Disc potency(μg)	Zone Diameter Interpretive Criteria(Nearest Whole Mm)		
		Susceptible	Intermediate	Resistant
Ampicillin	10	≥ 17	14-16	≤ 13
Amoxicillin	30	≥ 18	14-17	≤ 13
Cefoxitin	30	≥ 18	15-17	≤ 14
Chloramphenicol	30	≥ 18	13-17	≤ 12
Ciprofloxacin	5	≥ 31	21-30	≤ 20
Gentamicin	10	≥ 15	13-14	≤ 12
Kanamycin	30	≥ 18	14-17	≤ 13
Nalidixic Acid	30	≥ 19	14-18	≤ 13
Streptomycin	10	≥ 15	12-14	≤ 11

Annex 3: Plating and Biochemical Tests Record Sheet Format Used For *Salmonella* Isolation

Samples	Culture medias						Biochemical tests				
	BPW(pre-enrichment)	RVS	Selenite broth	Tetrathionate broth	XL D agar	Brilliant green agar	Indole test	TSI	Urease test	Simon's citrate test	MR-VP test



Annex 4: Flow diagram showing ISO method for detection of *Salmonella*

Source: ISO 6579 (2002)

