Isolation, Identificatin And Antimicrobial Resistance Profile Of *Salmonella* Isolated From Chicken Cloacal Swab In Asossa And Bambasi Town, Benishangul Gumuz Regional State

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Abstract: A cross - sectional study on isolation, identification and antimicrobial resistance pattern of *Salmonella spp* in Asossa and Bambasi town, Benishangul Gumuz Regional State were carried out from November 2017 to May 2018 with the objectives to estimate prevalence of salmonella species, associated risk factors and antimicrobial resistance pattern of the isolates. For this purpose, a total of 384 cloacal swab samples were collected and were subjected to various cultural and biochemical examinations. Among the 89 (23.2%) positive isolates, 7 fermented glucose and maltose and produced both acid and gas and did not ferment dulcitol which is positive for *S. pullorum*. 80(89.88%) of the positive isolates fermented glucose, maltose and dulcitol and produced acid, which are typical for *S.gallinarum*, two were S. typhimurium. Origin/sites/, age categories, body condition and sanitary/management condition were potential risk factors, which were statistically significant value for salmonella infection ($p<0.000$) whereas sex groups, floor type, previous treatment history and breed factors were not significant ($p>0.05$). Of the 89 isolates, 2.24 % were motile (contributes to zoonoses) while (97.75%) were non-motile. The antimicrobial susceptibility profile of all isolates were assessed against ten antimicrobials by disk diffusion technique; almost all isolates were resistant to one or more of the tested antimicrobials. Of all isolates, 95.6 % were multidrug resistant (MDR). 84.78%, 80.43%, 76.08%, 69.56%, 67.39%, 56.52% and 47.82% of the isolates were resistant to Tetracycline, Streptomycin, Kanamycine, Norfloxacin, Trimthoprim, Nalidixic Acid and Chloramphenicol respectively. However, the majority of the isolates were susceptible to ciprofloxacin and gentamycin, followed by sulphonamides. This is a significant threat to public health particularly to those who have direct or indirect contact to poultry and poultry products so that hygienic management of poultry and its products inorder to reduce the risk and selection of antimicrobials by antimicrobial sensitivity test were also suggested.
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1. Introduction

Salmonellosis is an important zoonotic disease caused by the genus *Salmonella* which constitutes a major public health burden and represents a significant cost in many countries. The prevalence of *Salmonella* in animals is a continuous threat to human health (Murugkar *et al.,* 2005). Salmonellae are widely distributed in nature and cause a spectrum of diseases in man, animal and birds. Poultry eggs, meat and their products are the commonest vehicles of *Salmonella* to humans (Nagappa *et al*., 2007). Every year millions of human cases are reported worldwide and the disease results in thousands of deaths (Herikstad *et al*., 2002).

Members of the genus *Salmonella* are Gram-negative, motile, facultatively anaerobic organisms belonging to the family *Enterobacteracae* (Ellermeier and Slauch, 2006). The genus *Salmonella* contains two species, *Salmonella enterica*, which consists of six subspecies, and *Salmonella bongori.* Currently the genus includes a total of more than 2,500 serotypes (Popoff *et al*., 2004). *Salmonella* nomenclature is complex, and is based on names for serotypes in subspecies I. For example, *Salmonella enterica* subsp. *enterica* serotype Enteritidis, is shortened to *S.* Enteritidis (Brenner *et al*., 2000). *Salmonella enterica* subspecies *enterica* (subspecies I) is responsible for 99.5 % of infection in man and animal (Martin *et al*., 2006). Most of the infections are zoonotic in origin but some serotypes like *S.* Typhi and *S.* Paratyphi infect only humans (Yan *et al*., 2003). The infectious dose, incubation period, symptoms and mode of transmission of salmonellosis caused by different serotypes are similar. Symptoms include diarrhea, fever and abdominal cramps with incubation periods ranging from 12 to 72 hours. The illness usually lasts from 4 to 7 days and most people recover without treatment. The elderly, infants and those with impaired immune systems are more likely to have a severe illness (Hans and Dean, 2006).

Salmonellosis is now a worldwide problem which istransmitted by faecal-oral route. It becomes the most important zoonotic disease because of its transmission route associated with contamination specifically via water and food. Early diagnosis of

salmonellosis using laboratory procedures and clinical result allows having time for applying a prevention strategy before the contaminated water or food entered tothe food chain. It also allows detecting outbreak early and treating patients (WHO, 2010).

The use of antibiotics in food animals selects bacteria which are resistant to antibiotics used in humans. These might be spread via the food to humans and cause human infection (Phillips, 2004). Amongst *Salmonella* spp., antimicrobial resistance is a well confirmed phenomenon and antimicrobial-resistant *Salmonella* are increasingly associated with the use of antimicrobial agents. Antimicrobials are substances that have significantly contributed to the prevention and treatment of infectious diseases in humans, as well as to many animal species (CDC, 2008). However, the excess or overuse of antimicrobials can generate genomic selective pressures to enable microbes to adapt and acquire resistance (Witte, 2001).

Ultimately, increases in bacterial antimicrobial resistance pose a considerable threat to public health, especially for vulnerable populations including young children (Shea, 2003), the elderly and immunocompromised individuals (Hitti and Wolff, 2005). Concentrated animal feeding operations (CAFOs) in agricultural practices have evolved to accommodate food consumption rates with increased agricultural output at the risk of introducing antimicrobial resistant pathogens into the environment. In addition, several studies have suggested that characteristics of agricultural environmental settings, including animal crowding, CAFO hygiene, temperature, ventilation control and stress, can influence antimicrobial resistance and pathogen risk (Silbergeld *et al*., 2008).

There are reports of high prevalence of resistance in *Salmonella* isolates from countries such as Taiwan (Lauderdale *et al.,* 2006), India (Mandal *et al.,* 2004, 2006), the Netherlands (Duijkeren *et al.,* 2003), France (Weill *et al.,* 2006), Canada (Poppe *et al.,* 2006) and Ethiopia (Ayalu *et al.,* 2011; Beyene *et al.,* 2011; Sibhat *et al*., 2011). The presence of resistant organisms in the poultry and poultry products for consumption is a safety concern to the population (Schlundt *et al.,* 2004) and therapeutic concern for the physicians which might pose prolonged treatment in cases of outbreaks, delayed recovery or treatment failure (Silbergeld, 2008). There is a scarcity of knowledge concerning poultry farm development associated with antimicrobial resistance and foodborne bacteria. Information on the antimicrobial resistance pattern of the *Salmonella* isolates from chicken table eggs could be useful for successful treatment, as well as planning strategic use of drugs to minimize resistance in the future.

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 presence of other diseases considered to be of high priority may have overshadowed the problem of salmonellosis (Oosterom, 1991).

In Benishangul Gumuz, poultry salmonellosis is endemic in local poultry farm as well as household farmstead and *Salmonella* species is recognized as a major cause of food borne illnesses, that are closely associated with the consumption of contaminated poultry and egg products, there is a desire to strengthen the monitoring and surveillance of salmonellosis using suitable diagnostic tools so as to prevent and control its occurrence. Besides this, the extent of *Salmonella* contamination of cloacal swab and antimicrobial profile of the *Salmonella* isolates has not been adequately studied and very limited information exists in the region and none in the Asossa and Bambasi Districts.

Therefore, the objectives of the current study are:

- To determine the prevalence of *Salmonellosis*
- To isolate and identify *Salmonella gallinarium/ pullorum* from local poultry farm
- To assess the risk factors associated with salmonella infections
- To determine the antimicrobial susceptibility patterns of the salmonella isolates

3. Materials And Methods

3.1 Description of the Study Area

The study was conducted in selected Asossa and Bambasi Districts of Asossa zone of Beni shangul Gumuz Regional state, from November 2017 to May 2018. Within districts it was studied in seven peasant association here after called namely: Asossa twon, Bambasi 02, Sonka, Mutsa, Mender 45, Mender 52, and Keshmando Pas of local poultry farm. Asossa zone has 214 peasant association, stretching over an area of 18,340.55 kilometer square, with human population of 270,980. The region is found in the north west of the country between latitude of 9 and 11^0 N and longitude of 34 and 35^0E and its altitude is from 700-1560 meter above sea level. Annual rain fall is between 900-1500 mm with uni modal type of rainfall that extends from April to October with peak rainy periods from June to August, and annual temperature ranges between 25- 35^0c (NMSA, 2014; CSA, 2015). Asossa zone, the livelihood of the society largely depends on mixed livestock and crop production. It has 35.6% of the livestock population of the region constituting 81,939 cattle, 167, 281 goats, 10,231 sheep, 14,089 donkeys, 40, 3153 poultry, 29 horses and 59,695 beehives (CSA, 2005; CSA, 2016).

Asossa district has 74 kebeles covering an area of 2317 km2 with human population of 47666. And also it is located between $8^0 30''$ and 4^{0}°27" N and 34°21" and 39°1" E. It has an altitude range of 1000-1570 meter above sea level and Its annual temperature ranges between 16^0c- 34^0c. Besides this, Bambasi district has 38 kebeles stretches over an area of 2210.16 square k.m with human population of 62693 and annual temperature ranges between 21^0c - 35^0c (CSA, 2015).

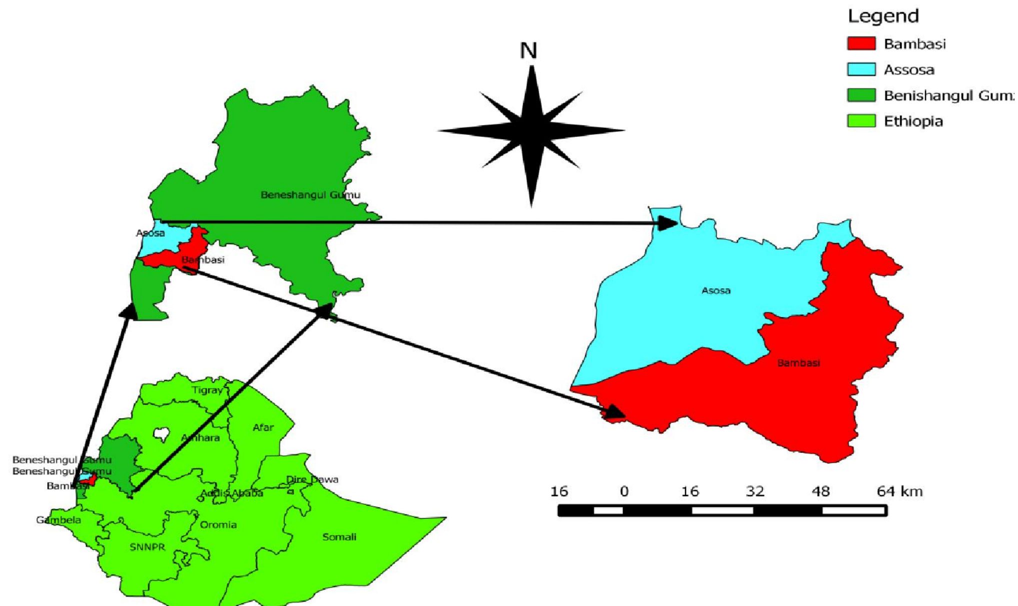


Figure 1: Map of the study area showing relative location of Assosa and Bambasi districts in Benshangul Gumuz Regional State

Source: (Mulaw *et al*., 2011)

3.2 Study Design

A cross - sectional study was carried out from November 2017 to May 2018 for isolation, identification and assess antimicrobial resistance profile of salmonella isolates from smallhouse hold local poultry farms.

3.3 Study population

The target population were apparently healthy chickens in local poultry farms of Bambasi and Asossa including local and exotic breed. A total of 384 commercial chickens and chickens of local poultry comprising different age group, management system, and breed and production level were included in this study. Birds are kept under semi intensive poultry management systems. Birds are provided with industrially produced poultry feed and water adlibitum.

3.4 Sample size determination

Total sample size for chicken cloacal swab sample collection, isolation and enumeration of salmonella species were assigned according to statistical formula of Thrustfield (2007). A 5 % absolute precision (5% sampling error) at 95% confidence interval was used during estimation of the sample size. Since there is no similar work done in the Asossa and Bambasi district, the expected prevalence was taken as 50% according to Thrustfield (2007). Therefore, the total sample size for the study were calculated using the following formula for each sampling units.

$n = \underline{(1.96)^2 x\ P\ (1-P)/}\ d^2$

Where: n = the total sample size, p = expected prevalence (50%), d = desired absolute precision/ marginal error between the samples and population / (5%), (0.05) at 95% CI,

$Z\alpha/2$ = the standard normal deviation corresponding 95% of confidence level = 1.96

n = (1.96) x (1.96) x (0.5) x (1-0.5)/ (0.05) x (0.05) = 384; accordingly, from a total of 384 chicken cloacal swab; 315 was sampled from local poultry farm at Bambasi and 69 swab sampled from Asossa local poultry farms.

3.5 Laboratory methods

3.5.1. Questionnaire survey

Data on each sampled chicken cloacal swab were collected using a properly designed questionnaire format for determining the associated risk factors. This includes enviromental contamination, management factor, feeding status, housing/ventilation/, treatment status, handling practices, chicken tansportation,

breed, age, sex, previous history of treatment, bio security measures, hygienic/ sanitary condition and other relevant information related to salmonellosis was gathered.

3.5.2 Sampling methods, collection and transportation of samples

Purposive sampling technique was applied for selection of study sites, based on the availability of chickens, accessibility and presence or absence of disease in kebeles. Besides, random sampling methods was used for selection of each chicken in Asossa and Bambasi local poultry farmstead. A total of 384 cloacal poultry swab samples were collected aseptically from every local poultry farms. Aseptic procedure were followed when collecting samples. The sterile plastic bags or cotton bud/ sterile ice box/ were used for containing selected cloacal swab. The cloaca/ vulva/ surface was sterilized by swabbing in 70 % alcohol for 2 min. The cloacal swab samples were collected in sterile ice box. The collected swab samples from Asossa and Bambasi poultry farmsteads were individually placed into a sterile plastic container in an ice box. Therefore, samples were properly transported immediately in an ice box to the analyzing Regional Veterinary Laboratory of Benishangul Gumuz, Asossa, for microbiological examination. The isolation was conducted utilizing the conventional methods for the detection of *Salmonella species* following the standard guidelines from ISO 6579 (ISO, 2002).

3.5.3 Cultural isolation techniques

According to the International Organization for Standardization (ISO 6579, 1998) it is customar to use three stage processes: pre-enrichment, selective enrichment and selective plating to isolate *Salmonella.*

3.5.3.1 Pre-enrichment in non-selective liquid medium

Pre- enrichment allows the resuscitation and multiplication of sub-lethally damaged *Salmonella* cells (Blackburn, 1993). Non-selective media such as buffered peptone water (BPW) and nutrient broth are most widely used for resuscitation; buffered peptone water being recommended for routine purposes. BPW inoculated at ambient temperature with the test portion, then incubated at 37 °C ± 1 °C for 18 h ± 2 h. For large quantities, the buffered peptone water should be heated to 37 °C ± 1 °C before inoculation with the test portion. The need for resuscitation is now widely accepted for all types of samples and not merely those which have been dried or frozen (Varnam and Evans, 1991).

3.5.3.2 Enrichment in selective liquid media

Selective enrichment helps to increase the ratio of *Salmonella* to competitor organisms. Many types of inhibitors have been proposed for the selective enrichment of *Salmonella,* the most widely used of which bile, tetrathionate, selenite and dyes are including brilliant green and malachite green. Various formulations of selenite and tetrathionate broths have been widely used, although in recent years there has been increasing use of the malachite green based Rappaport-Vassilliadis medium with soya (RVS) broth, the RVS broth is incubated at 41.5 °C ± 1 °C for 24 h ± 3 h (Varnam and Evans, 1991; Blackburn, 1993).

3.5.4 Plating out and identification of salmonella spp

Plating on selective agar media enables the recognition of *Salmonella* colonies while suppressing the growth of the back ground microflora. A wide range of media has been devised for selective plating. Selective plating media for *Salmonella* all contain a diagnostic system to permit differentiation of the organisms from non-*Salmonella*. This is commonly based on the inability of most salmonellas to ferment lactose and, in some cases, other carbohydrates such as sucrose and salicin. Bile containing media often employ a second diagnostic system based on the ability of *Salmonella* to produce hydrogen sulphide. Where competition from other bacteria is insignificant, a general-purpose medium such as MacConkey agars may be used (Quinn *et al*., 2002).

Cloacal swabs were collected by sterile cotton and the swabs with bud were immediately inoculated in to nutrient broth incubated at 37°c for 1-2hrs and /or the RVS broth is incubated at 41.5 °C ± 1 °C for 24 h ± 3 h. In many cases, greater selectivity is required and it is necessary to use a medium devised specially for *Salmonella*, such as brilliant green agar (BGA), *Salmonella-Shigella* agar (SS agar), Xylose- lysine deoxycholate agar (XLD agar) and Eosin- methylene blue agar (EMB) agar plate were used for plating and identification purpose (Varnam and Evans, 1991; Blackburn, 1993; Quinn *et al*., 1994). So, the nutrient broth containing the samples were incubated at 37° C for 1-2 hrs. A loop-full of inoculum from each cloacal swab sample was transferred and streaked/ spread/ separately onto the surface of S-S agar plates. The plates was incubated at 37 ^{o}C ± 1 ^{o}C for 24 ± 3 hours. After proper incubation, the plates were examined for the presence of suspected *Salmonella* colonies, which on SS agar were colourless or translucent and black color colonies were observed. The pure organisms were sub- cultured in to XLD agar were pink with a darker center and a lightly transparent zone of reddish color due to the color change of the indicator whereas lactose positive salmonellae are yellow with or without blackening. Similarly, subcultured onto EMB agar, small, circular pink colour colony was examined. Thus single pure colony was obtained. These pure isolates were used for the further study. 89 *Salmonella* presumptive colonies were transferred to non selective

solid media for further confirmatory tests. Confirmation was done by using biochemical test according to ISO 6579 (ISO, 2002).

3.5.3.1 Gram's staining

The colonies showed black in color were subjected to subculture in S-S agar to make pure colony which were further used for Gram's staining. In Gram's staining the organism appeared as gram negative rod under light microscope. The organisms were sub-cultured into EMB Agar and XLD Agar. These pure salmonella colonies/ isolates/ were characterized morphologically using Gram's stain according to the method described by Merchant and Packer (1967). Briefly, a small colony was picked up from SS agar with a bacteriological loop, smeared on separate glass slide with a drop of distilled water and fixed by gentle heating. Crystal violate (Hi-media, India) was then applied on each smear to stain for two minutes followed by washing with running water. Few drops of Gram's Iodine (Hi-media, India) was then added, which acted as mordant for one minute and then washed with running water. Acetone alcohol (Hi-media, India) was then added (acts as decolorizer) for few seconds. After washing with water, safranin was added as counter stain and allowed to stain for two minutes. The slides were then washed with water, dried in air and then examined under light microscope with high power objective (100X) using immersion oil (Quinn *et al*., 2002).

3.5.4 Differentiation of Isolated salmonella by biochemical tests

For this study isolated organisms with supporting growth characteristics of *Salmonella* were subjected to sugar (Carbohydrate) fermentation test, Triple sugar iron agar (TSI), Urea agar, Simmon's citrate, Lysine iron agar, MR-VP reaction, indole reaction and motility.

3.5.4.1 Sugar (Carbohydrate) fermentation test

The carbohydrate fermentation test was performed by inoculating a loopful of thick test bacterial culture into the individual tubes containing sugars like dextrose/glucose/, maltose, lactose, dulcitol and incubated at 37^0 C for 24 hours. Acid production was indicated by the change of media from pink to yellow color while gas production was indicated by the appearance of gas bubbles in the inverted Durham's fermentation tubes.

3.5.4.2 Triple Sugar Iron (TSI) Agar

The Triple sugar iron agar slants were prepared with a thick butt. A loopful culture of pure growth from nutrient agar was stabbed into the butt and streaked on the slant and was incubated for 24 hours at 37 ^{o}C. Typical *Salmonella* cultures show alkaline (red) slants and acid (yellow) butts with gas production (bubbles) and formation of hydrogen sulfide (blackening of the agar) (ISO 6579:2002(E); Quinn *et al*., 2002).

3.5.4.3 Urea agar

The hydrolysis of urea releases ammonia and production of ammonia increases the pH of the medium that change color of phenol red (pH indicator) to rose pink, and later to moderate red. The basal medium was sterilized by autoclaving at 121 ^{o}C for 15 minutes. When it has cooled to about 50 ^{o}C, 100 ml of a 20 percent solution of pure urea previously sterilized by filtration was added and poured into test tubes. The isolates were inoculated into the urea to determine urease production. The inoculated tubes can be incubated at 37 ^{o}C for up to 96 hours. The observations may be made at an interval of 4, 24, 48 and 96 hours. Urease positive cultures changed the color of the indicator to red (ISO 6579:2002(E); Quinn *et al*., 1999).

3.5.4.3 Simmon's Citrate utilization test

Simmon's citrate agar was sterilized by autoclaving at 121 °C for 15 minutes at 15 lb pressure and cooled for slant formation. The strains were cultured on the prepared Simmon's citrate agar medium, incubated at 37 °C for 48 hours and observations were recorded. Opacity and change in color of bromothymol from green to blue indicated a positive reaction (Quinn *et al*., 2002).

3.5.4.4 L-lysine decarboxylation medium/ Lysine iron agar/

Lysine - decarboxylation broth was inoculated with the loopful culture of the test organism and one was kept uninoculated control. Both tubes was incubated for 24 hours at 37 ^{o}C. Turbidity and a purple color after incubation indicate a positive reaction. A yellow color indicates a negative reaction (ISO 6579:2002(E); Quinn *et al*., 1994).

3.5.4.5 Indole test

Indole is a nitrogen-containing compound that can be formed from the degradation of the amino acid tryptophan by certain bacteria. Tryptone was used as a substrate because it contains much tryptophan. The indole reacts with aldehyde compound of kovac's reagent and forms red coloured compound that is more soluble in alcohol. For indole test peptone water was prepared and the ingredients were dissolved in distilled water, dispensed in test tubes and sterilized by autoclaving at 121 ^{o}C for 15 minutes. The tubes of the medium were inoculated with test isolates using sterile platinum loop and incubated at 37 ^{o}C aerobically for up to 96 hours. Finally, 0.5 ml of kovac's reagent was added to each of the inoculated and un inoculated controls. The tubes were shaken gently and the results were recorded. Positive results were indicated by the development of red colour in the alcoholic layer of the reagent and no colour in the control tube (Quinn *et al*., 2002).

3. 5.4.5 Voges-Proskauer (VP) test

Two ml of sterile glucose phosphate peptone broth were inoculated with a pure colony of test organisms and incubated at 37^0C for 24 hours. A very small amount (knife point) of creatine was added and mixed and 3 ml of sodium hydroxide were added and shaked well. The bottle cap was removed and left for an hour at room temperature. It was observed closely for the slow development of a pink colour for positive cases.

3.5.4.6 Methyl-Red (MR) test

The test was performed by inoculating a colony of the test organism in 0.5 ml sterile glucose phosphate broth. After overnight incubation at 37^0C, a drop of methyl red solution was added. A positive methyl red test was shown by the appearance of bright red colour indicated acidity while a yellow or orange colour was considered as negative.

3.5.4.7 Motility test

The motility test was performed to differentiate motile *Salmonella* from non-motile one. This test was performed in Motility Indole Urea (MIU) medium (Hi-media, India), where a sterile straight wire used to inoculate 5 ml of sterile MIU medium taken earlier in a screw caped test tube with a smooth pure colony of the test organism. When inoculating the MIU medium, a stab was made with a sterile straight wire and stoppered the tube followed by incubation at 35-37° overnight. Motility is shown by a spreading turbidity from the stabline or turbidity throughout the medium (compared with an uninoculated tube).

3.6 Antimicrobial Susceptibility test

The *Salmonella gallinarium or Salmonella pullorium* isolates was tested for anti-microbial susceptibility by disc diffusion method (Quinn *et al.,* 2002). The antibiotics that were used against the isolated organisms with their disc concentration are Chloramphenicol 30 µg (CHL), Ciprofloxacin 5 µg (CIP), Streptomycin (10µg), Gentamycin 10 µg (GEN), Kanamycin 30 µg (KAN), Tetracycline 30µg (TE), Norfloxacin 10 µg (NOR), Nalidixic acid (NA) (30µg), and Trimethoprim 5 µg (TMP), Oxoid Company (Hampshire, England), was used for Anti-microbial susceptibility testing.

Approximately 3-5 Colonies isolated from pure culture was transferred into a test tube of 5 ml peptone/ nutrient broth/ and suspension was made and incubated at 37^oc for 8 hours. The turbidity of the suspension was adjusted by adding 9ml saline water and/ or the turbidity of the suspension was adjusted comparing with that of 0.5 McFarland standards. Muller-Hinton Agar plate was prepared and a sterile cotton swab was dipped into the suspension and swabbed on the surfaces of Muller-Hinton Agar plate. Then, the antibiotic discs was placed on the agar plate using disc dispenser/ sterile forceps and pressed gently to ensure the complete contact with the agar surface. The plates was read after 24 hours of incubation at 35^0C under aerobic condition. The isolates was classified in accordance with the guideline of the National Committee for Clinical Laboratory Standards (CLSI, 2006) as susceptible, intermediate or resistance for each antibiotic tested according to the manufacturer's instructions by measuring, the diameter of the zone of inhibition around the antibiotic disc. This method allowed for the rapid determination of the efficacy of the drugs. Intermediate results was considered as resistant (Huber *et al.*, 2011). Multiple antibiotic resistant (MAR) phenotypes was recorded for isolates showing resistance to three and more antibiotics (Rota *et al.*, 1996).

3.7. Data Management and Statistical Analysis

Processing of data was done by computer software. Data was coded and entered to MS Excel spreadsheet and checked for accuracy. After validation, it was transferred and processed using computer software STATA version 11 for analysis. Pearson's chi-square tests were used when appropriate to analyze the proportions of categorical data. Odd ratio and 95% CI were computed, the 95% confidence level was used, and results was considered significant at ($P < 0.05$).

4. Results

4.1 Prevalence of Salmonella in poultry

Table 5**: Origin based prevalence of salmonella by culture and biochemical test**

Study sites	No of chickens examined	Prevalence%	Chi2	p-value	OD	95 % CI
Bambasi no. 02	61	22(36.06)	15.59	0.02	1.06	-0.01-0.03
Sonka	57	6(10.52)				
Mutsa	49	9(18.36)				
Mender 45	48	11(22.91)				
Mender 52	59	10(16.94)				
Keshmando no.2	41	14(34.14)				
Assosa town	69	17(24.63)				
Total	**384**	**89(23.2)**				

A total of 384 local and exotic poultry samples were randomley collected at Bambasi and Assosa districts during the study period. Samples were processed microbiologically for isolation and identification of *Salmonella*. Based on the bacteriological culture and biochemical test, 89/ 384 (23.2%) *Salmonella spp* were isolated and it was found to be statistically significant (P<0.02, Chi2=15.6). The highest salmonella distribution were observed in Bambasi 02 (36.06%) while the lowest prevalence was reported in Sonka (10.52%) as shown in (Table 5).

(384 Chicken swab samples were examined, overall salmonella prevalence at different study site level were (n=384, 23.2%), by bacteriological method. The prevalence of salmonella amongst study sites has significant difference (df= 6, X^2=15.59, Pr=0.016) (Table 1).

4.2. Isolation of Salmonella by cultural characteristics

4.2.1 Results of culture in different culture media

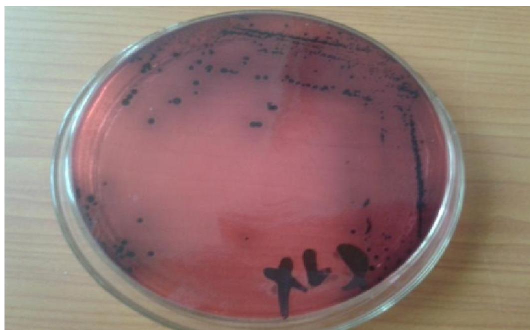


Fig-1. Growth of *Salmonella gallinarium* colony on XLD agar

RVS culture, showed two types of colony morphology; isolates showed colorless, translucent, smooth and raised colonies on MLA, indicative of lactose non fermenter organisms and others produced pink color colonies indicative of lactose fermenter organisms. On XLD agar, red colonies were produced initially after 24 h of incubation, which get blackened at center on prolonged incubation (Fig-1). Isolated salmonella spp were also grown on S-S agar all of the isolates were produced translucent, black, smooth, small round colonies which are positive for *Salmonella* (Fig-2), Salmonella suspected colony were inoculate to EMB agar and produced pink color colony on EMB agar (Fig.3). On BGA, non lactose fermenter isolates produced light pink colony against a rose pink background (Fig-4) and the grown isolate were subjected to sub culturing for pure colony appreciation and after each bacterial colony was characterized, identification of bacteria through primary and secondary biochemical tests was conducted.

4.2.1.1 Xylose -Lysine Deoxychockolate (XLD) agar media

All of the suspected Salmonella isolates produced pink color colony with black centre.

4.2.1.2 Salmonella-Shigella agar

On S-S agar, all of the isolates were produced translucent, black, smooth, small round colonies which are positive for *Salmonella*.

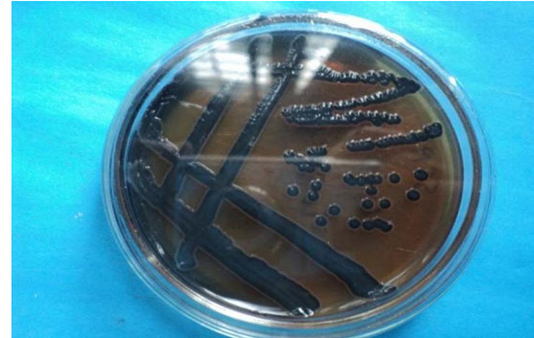


Fig-2. *Salmonella* colony on S-S agar

4.2.1.3 Eosin - Methylene Blue (EMB) agar media

Salmonella suspected colony were inoculate to EMB agar and produced pink color colony on EMB agar.

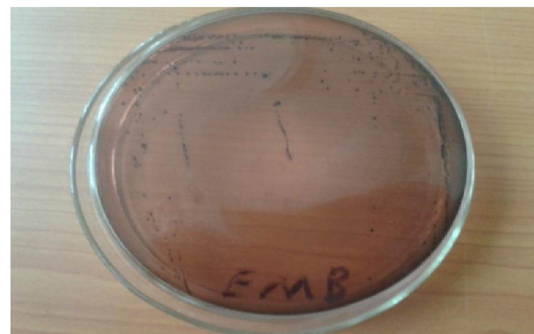


Fig-3: Cultural properties of *Salmonella* on EMB agar.

Fig- 4. Growth of salmonella on BG agar

4.3 Results of Gram's staining technique

The microscopic examination of Gram's stain revealed Gram-negative, pink colored, short rod shaped bacteria, arranged singly and in pair.

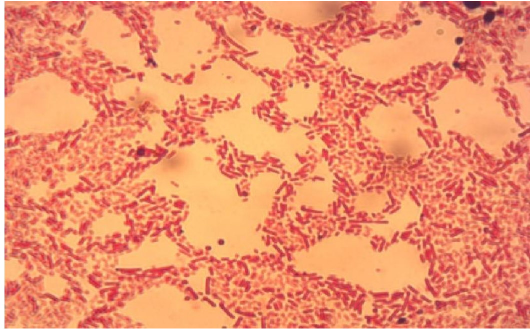


Fig-5: Gram's staining of Salmonella isolates (scale bar 10X100), Gram-negative, pink colored, short rod shaped bacteria

4.4. Biochemical profile of bacterial isolates

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, Lysine iron agar/LIA/, Simmon's citrate agar, urea broth, MR-VP broth and incubated for 24 or 48 hours at 37 ^{o}C. Colonies producing an alkaline slant with acid (yellow color) butt on TSI with hydrogen sulphide production, positive for lysine (purple color), negative for urea hydrolysis (red color), negative for tryptophan utilization (indole test) (yellow-brown ring), negative for voges-proskauer, positive for methyl red test and positive for citrate utilization, catalase positive and oxidase negative were considered to be *Salmonella*-positive (ISO 6579, 1998; Quinn *et al.,*2002).

The results from the multiple biochemical tests are illustrated in the following figures.

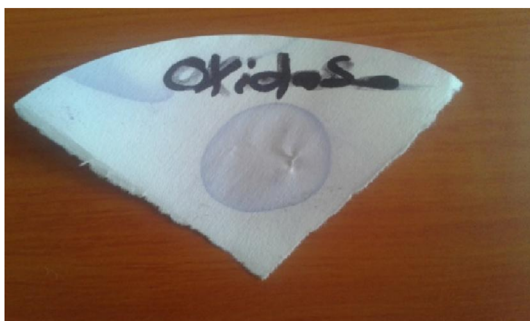


Figure 6: Oxidase test negative of *S. gallinarium*

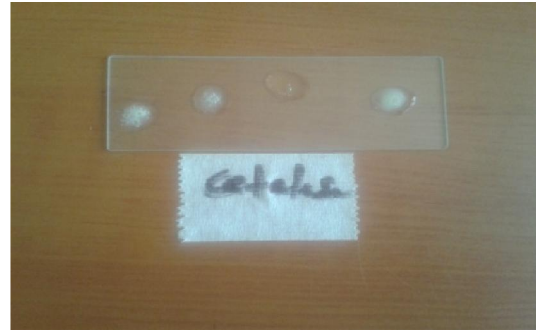
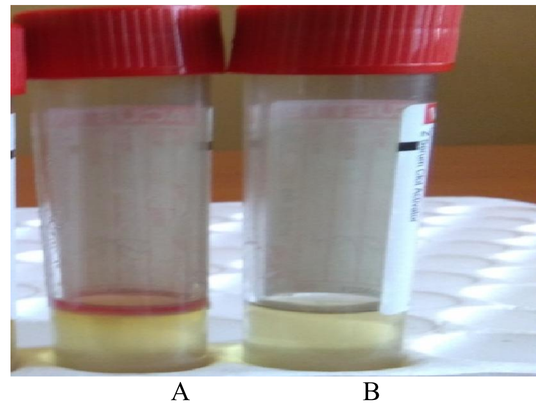


Figure 7: positive catalase test of *S. pullorum/ S.gallinarium/*

Figure 8: R/Y/H_2S^+ on TSI shown *salmonella spp*



A B

Figure 9: Indole positive test shows red ring formation at the top of SIM broth (A) where as Indole negative is indicated in (B).

Fig- 10. Urease test positive test shows red formation at the slant (A) where as urease negative is indicated in (B).

Fig-11. Simone's citrate test positive test shows blue colour formation at the slant (A) where as citrate negative is indicated in (B).

Fig-12. Lysine iron agar (LIA) test positive test shows purple colour formation at the slant (A) where as lysine negative is indicated in (B).

In the following, indole negative isolates, Methyl Red test positive isolates, and all isolated salmonellae were negative for VP test isolates were indicated, as shown in fig.13.

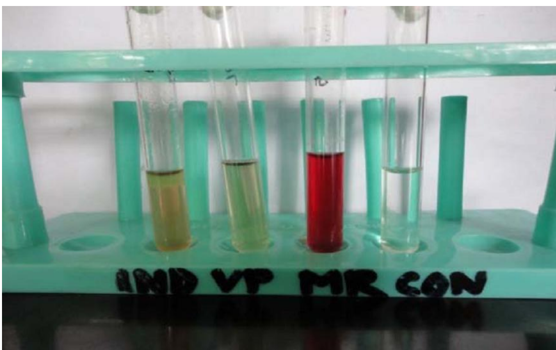
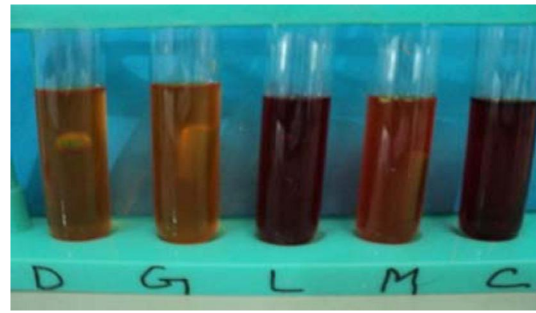


Fig-13: Results of biochemical test.

4.4.4 Carbohydrate fermentation reaction

Among the 89 positive isolates 7 were fermented glucose and maltose and produced both acid and gas and did not ferment dulcitol which is positive for *S. pullorum*. 80 of the isolates fermented glucose, maltose and dulcitol with producing acid, which are typical characteristics for *S. gallinarum*. Acid production was marked by the color change from reddish to yellow. Gas production was marked by accumulation of gas in the Durham's tube. It was observed after incubation at 37^0C for 48 hours.



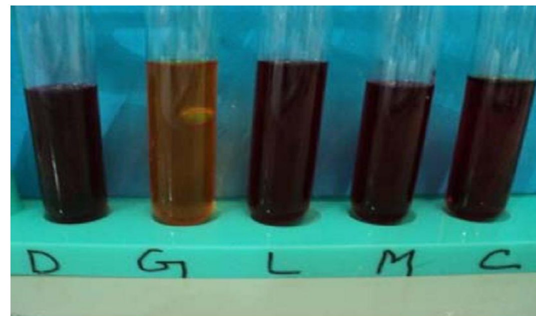


Fig-14: Sugar test indicating *S. gallinarum/S. typhimurium*

Fig-15: Sugar test indicating *S. pullorum.*

Figure 16: maltose positive (yellow) whereas maltose negative (red, unchanged)

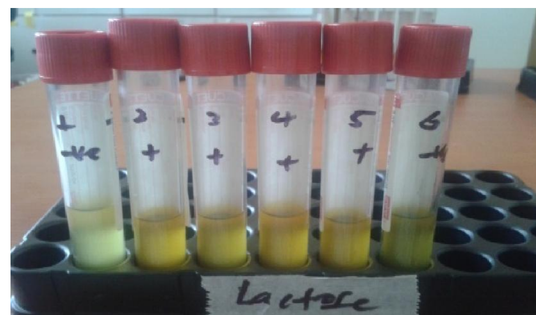


Figure 17: Lactose positive (yellow)

Figure 18: Glucose positive (yellow)

In general, Dulcitol, glucose and maltose positive, acid production with out gas and negative for lactose was *Salmonella gallinarium* whereas acid, gas production and non lactose fermenter was said to be S. pullorium. 80 positive was *S. gallinarium*, 7 positive was S. pullorium and 2 positive was S***almonella typhimurium***. So as this study indicated most of the sugar test was showing salmonella gallinarium than S. pullorium as shown in the figures.

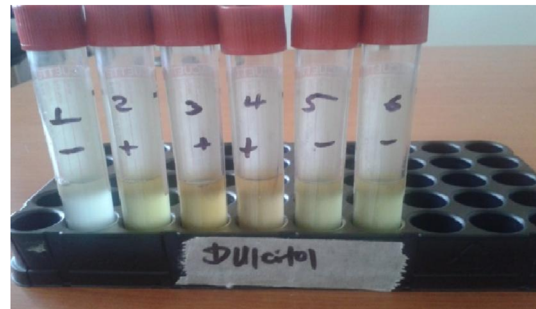


Figure 19: Dulcitol negative while positive (yellow)

Table-6: Results of different biochemical tests.

Tests	*Salmonella Pullorum*	*Salmonella Gallinarum*	*Salmonella Typhimurium*
Lactose fermentation	-	-	-
Glucose	+ (Acid and Gas)	+ (Acid)	+ (Acid and Gas)
Dulcitol	-	+ (Acid)	+ (Acid)
Maltose	± (Acid and Gas)	+ (Acid)	+ (Acid and
Indole Production	-	-	-
Methyl red test	+	+	+
Voges-Proskauer test	-	-	-
Motility	-	-	+
Total positive isolates	7	80	2

4.4.5 Frequency results of motility tests

Among 89 isolates, 87 isolates were found to be non motile characterized by forming the stab line with out producing turbidity in the motility indole, SIM medium, and another 2 isolates were found motile characterized by changing of the colour of SIM medium. All 89 (23.2%) positive isolates were screened for motility test. 87 (97.75%) isolates were found non motile while 2 (2.24%) were motile, which were isolated from cloacal swab, as shown in table 7.

Table 7: Motility test for positive isolates

Motility test	Total	No. of positive sample test	Prevalence (%)
Non- motile	89	87	97.8
motile		2	2.24

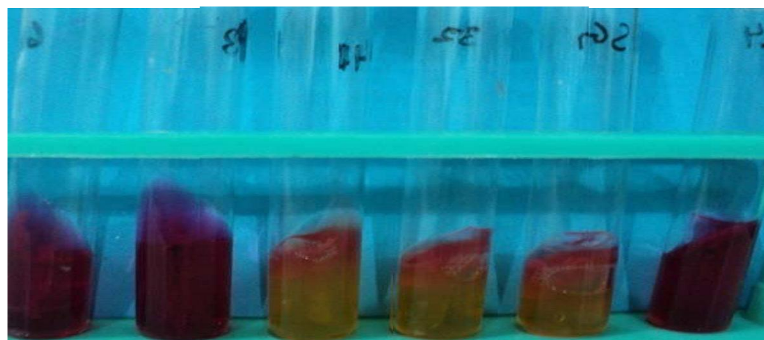


Fig 20: MIU/SIM media showing motile and non motile salmonella

4.3. Risk Factors Associated with salmonella Prevalence

Prevalence of salmonella related to the specific risk factors were determined as the proportion of affected chickens out of the total examined. As indicated in (Table 8), the questionnaire survey and observation data result shows age, body conditions, management/ sanitary status are amongst the potential risk factors, which are associated with salmonella disease in poultry/chicken farmstead. Accordingly, salmonella prevalence showed significant variation among different age groups (p = 0.002), body conditions status (p=0.000), sanitary and management status (p=0.000). However, breed, sex, floor, and previous treatment status have no significant difference with salmonella (p>0.05) as indicated in table 8.

Table 8: Distribution of total *S. gallinarium/S. pullorum/* in poultry population in Assosa and Bambasi "woreda" association with different potential risk factors

Factor	Level	No examined	Prevalence (%)	X^2	P-value
Age	1-4m	128	43 (33.59)	12.01	0.002
	>4-7m	182	31(17.03)		
	>7m-2yr	74	15 (20.27)		
Sex	Male	164	44(26.82)	2.14	0.14
	Female	220	45(20.45)		
Bcs	Good	162	27(16.66)	71.78	0.000
	Medium	155	20(12.90)		
	Poor	67	42(62.68)		
Sanitary	Good	160	10(6.25)	44.13	0.000
	Poor	224	79(35.26)		
Housing system	floor bedding	290	73(25.17)	2.64	0.10
	cage system	94	16(17.02)		
Previous treatment	Yes	162	36(22.22)	0.14	0.705
	No	222	53(23.87)		
Treatment status	Yes	133	32(24.06)	0.08	0.76
	No	251	57(22.70)		
Management status	Good	142	17(11.97)	22.13	0.000
	Medium	76	15(19.73)		
	Poor	166	57(34.33)		
Breed	Local	152	28(18.42)	3.19	0.074
	Exotic	232	61(26.29)		

4.4. *In vitro* antimicrobial Susceptibility Test

From a total of 89 isolates of *S.gallinarium/S. pullorum/* obtained from the study antimicrobial susceptibility tests were performed on 46 isolates. Due to the relatively small size, no separate analysis was undertaken for clinical isolates of *S. gallinarium* and were tested for antimicrobial sensitivity for 10 different types of antibiotics. The present study has demonstrated the existence of the levels of resistance of *S. gallinarium* to commonly used antimicrobial agents in the study area. The antimicrobial susceptibility profile of all isolates were assessed against ten antimicrobials by disk diffusion technique; almost all isolates were resistant to one or more of the tested antimicrobials. 84.78%, 80.43%, 76.08%, 69.56%, 67.39%, 56.52% and 47.82% of the isolates were resistant to Tetracycline, Streptomycin, Kanamycine, Norfloxacin, Trimthoprim, Nalidixic Acid and Chloramphenicol respectively (Table-9). However, the majority of the isolates were susceptible to ciprofloxacin and gentamycin, followed by sulphonamides (Table-9).

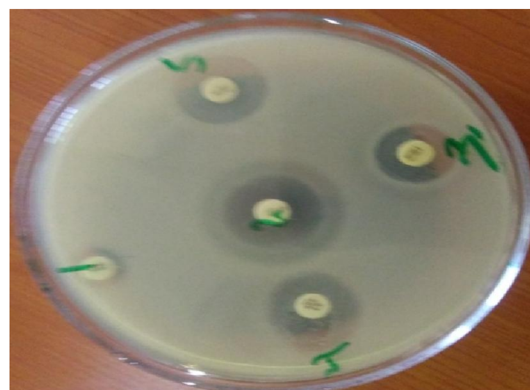


Figure 8: Antimicrobial drug sensitivity test on sample from Assosa and Bambasi districts. 1) TTC, 2) Sterptomycin, 3) Norfloxacin, 4) Gentamycin, 5) Kanamycin

Out of 46 isolates, 44(95.65%) were /MDR/ resistant to different combinations of two or more/ multidrug/ tested antimicrobials and the remaining 1(2.2%) isolates were non multidrug resistance/non MDR/. Besides this, 6 (13.04%) of the isolates were the most frequent multidrug resistant pattern to four drugs which were, Tetracycline, Streptomycin, kanamycin and norfloxacin as shown in table 10. From the total pure isolated *S. gallinarium,* 2(4.4%), 3(6.5%), 4(8.7%), 7(15.2%), 8(17.4%), 9(19.6%), and 12(26.08%) of the isolates were resistant for 2-10 drugs, respectively in Table 10.

Table 9: Antimicrobial susceptibility test result for Salmonella isolates (n = 46).

Antimicrobial agents	**Disc content (µg)**	**No of isolates**	**Resistance**	**Intermediate**	**Susceptible**
			No (%)	No (%)	No (%)
Tetracycline (TE)	30	46	39 (84.78)	3 (6.52)	4 (8.69)
Gentamycin (CN)	10	46	8 (17.39)	12 (26.08)	26 (56.52)
Streptomycin (S)	10	46	37(80.43)	4 (8.69)	5 (10.86)
Chloramphenicol (C)	30	46	22(47.82)	10(21.73)	14(30.43)
Trimethoprim (w)	2	46	31(67.39)	4(8.69)	11(23.91)
Kanamycin (K)	30	46	35(76.08)	6(13.04)	5(10.86)
Sulphonamides (S3)	300	46	19(41.30)	3(6.52)	24(52.2)
Nalidixic Acid (NA)	30	46	26(56.52)	12(26.08)	8(17.39)
Ciprofloxacin (CIP)	5	46	3(6.52)	-	43(93.47)
Norfloxacin (NOR)	10	46	32(69.56)	6(13.04)	8(17.39)

Key: S- Susceptible, I- Intermediate, R- Resistant profile of *Salmonella* isolated from cloacal swab of chicken.

Table 10: **Multi drug resistances (MDR) profile of the isolated *Salmonella***

Number	**Antimicrobial resistance pattern (No.)**	**No. of isolates resistance (%)**
One	GEN (1), S (1)	2(4.34)
Two	CAF & STR (1)	1(2.17)
Four	STR, NAL, TET & GEN (1)	8(17.39)
	KAN, S, TET & NOR (6)	
	S3, CAF,TET, CIP (1)	
Five	STR, KAN,NAL,TMP, TET (2)	7(15.22)
	STR, KAN, NAL,TMP,TET (1)	
	KAN, S3, NAL,W,TET (4)	
Six	STR,CAF,NAL,TET,GEN, & NOR (1)	6(13.04)
	STR,S3,NAL,NOR,TET & CAF (2)	
	STR, KAN,NAL,TMP,NOR,TET (3)	
Seven	KAN,S3, NAL,TMP,NOR,TET & CAF (1)	4(8.69)
	STR,KAN,NAL,TMP, CAF,NOR,TET (3)	
Eight	STR,CAF,KAN,NAL,TMP,TET,GEN & NOR (4)	9(19.56)
	STR,CAF,KAN,S3,GEN,TMP, NOR & TET (1)	
	STR,CAF,KAN,S3,NAL, NOR,TET & TMP (3)	
	STR,CAF,KAN,NAL,TMP,GEN,TET & S3 (1)	
Nine	STR,CAF,KAN,NAL,TMP,NOR,GEN,TET & S3 (1)	6(13.04)
	CAF,KAN,S3,NAL,TMP,NOR,GEN,TET & CIP (3)	
	STR,CAF,KAN,S3,NAL,TMP,TET,CIP, & GEN (2)	
Ten	STR,CAF,KAN,S3,NOR,NAL,TMP,GEN,TE & CIP (1)	3(6.52)
	STR,CAF,KAN, S3, NAL,TMP,NOR, GEN, TET & CIP (2)	
Total		**46(100%)**

Key: GEN = Gentamicin; KAN = Kanamycin; CIP = Ciprofloxacin; CAF = Chloranphenicol; TMP = Trimethoprim; S3 = Sulphonamides; TET = Tetracycline; NAL = Nalodixic Acid; and STR = Streptomycine, NOR= norfloxacin

MAR (Multiple Antibiotic Resistance) Index

The MAR Index of an isolate is defined as a/b, where a represents the number of antibiotics to which the isolate was resistant and b represents the number of antibiotics to which the isolate was subjected. The MAR indexes of the isolates were calculated and

noted and if bacteria having MAR Index > 0.2 indicates originate from an environment where several antibiotics are used (Jayaraman *et al*., 2012).

In present observation, number of drugs to which the isolate was resistance = 7, number of antibiotic to which the isolate subjected =10, so MAR index = 7/10= 0.7; so, the MAR Index analysis reveals that the isolate had a very high MAR index value (>0.2).

5. Discussion

5.1. Prevalence of salmonella in poultry

In the present study, the overall prevalence of salmonella was 23.2 % in chickens/ poultry, which was statistically significant and associated with the infection (Chi=15.59, p< 0.02). This result was in line with the previous studies made by Kasech M. (2015) in DAZARC poultry farm, at Bishoftu, Central Oromia, by Beshatu F. (2014) in Dire Dawa municipal abattoir, Ashwani *et al*. (2014) by serology method in Ethiopia, 30.4 %, 18% and 20% salmonella infection respectively. Besides this, the prevalence of *Salmonella* in chicken samples was concord with the results of earlier studies made by Molla *et al*. (1999a) who reported 28.6%, 22.6%, and 15.4% in chicken gizzard, liver, and heart, respectively, Molla and Mesfin (2003) who detected (21.1%) *Salmonella* in chicken carcass and giblets samples in central Ethiopia, Tibaijuka *et al.* (2003) who indicated 18 % prevalence (54/301) in chicken meat and edible offals and Hang'ombe (1999) who published 20.5% frequency of isolation for *Salmonella* from dressed chicken carcass in Lusaka, Zambia. This variability in prevalence of salmonellosis between different reports could be attributed to differences in farms management practice. As poultry salmonellosis is a complex disease involving interactions of various factors such sanitary problems, environmental conditions, and causative agents, contamination in the farm during collection, transportation and poor hygiene of workers as well as farms and different in different farming system. Different authors reported that the presence of chickens of different ages in the farm, the presence of arthropod pests, wet and soiled litter in the farm (Smeltzer T *et al*., 1979) and the housing system and flock size could be important reasons for egg contamination with various micro-organisms. Chicken feeds and hatcheries also possible sources of *Salmonella* infections in the farm.

However, this finding is higher as compared with the previous findings of Solomon T *et al*., 2016) in Alage, Ziway and Shashemene area, Endrias ZG (2004) in Addis Ababa supermarkets, Liyuwork T *et al*. (2013) in Addis Ababa and F. Abunna *et al*. (2016) in and around Modjo, Central Oromia, (Aseffa *et al*., 2011) from chicken table eggs by bacteriological methods in Ethiopia, (Hassanain *et al*., 2012) in Egypt, and (Urji *et al*., 2005) in Nigeria by bacteriological methods, 13.3%, 14%, 1.6%, 15.2%, 11.5%, 11.4%, and 12.5% salmonella infection in poultry farm respectively. The difference might be difference in farming system, poor hygienic practice in semi-intensive farm might contribute the major problem for high prevalence rate of salmonellosis.

Higher prevalence than present finding was also reported in Ethiopia and in other counties as 41.9% (Kindu and Addis, 2013) from fecal sample by bacteriological method, 35.7% (Endris *et al*., 2013) of *S. Gallinarum* and *S. pullorum* from cloacal swab by serology and culture, 55% (Kagambega *et al*., 2013) in Burkina Faso, 56.5% (Khan *et al*., 2014) in Pakistan, 45% v 60% (Jahan *et al*., 2012) in Bangladesh in cloacal poultry swab samples, 66% (Jerngklinchan *et al.* 1994) from Thailand, 29.7% by Plummer *et al.* (1995) from whole bird in UK, 38.3% (Rusal *et al*., 1996) in Malaysia from poultry carcass arising from wet markets and processing plants, and Arumugaswamy *et al., (*1995) from Malaysia also reported a much higher *Salmonella* isolation rate from chicken portions (39%), liver (35%) and gizzard (44%).

Likewise, lower prevalence than the present finding was also reported in Ethiopia and other countries. Few examples include 0.8% (Kassaye *et al*., 2010) of *Gallinarum* and *S. pullorum* from cloacal swabs by culture technique, 10.9% (Agada *et al*., 2014) in Nigeria, 9.2% (Al-Abadi and Al-Mayah, 2012) in Iraq in culture techniques in cloacal swab samples and 32(16%) of the 198 skin samples (Whyte *et al*. (2000) in Ireland, using the culture methods. These differences above (higher or lower prevalence) from present finding might be resulted from the difference in isolation technique, and difference in geographical location, difference in biosecurity measure like cross – contamination and poor housing system.

5.2 Effects of potential risk factors on the occurrence of S. gallinarium

Occurrence of salmonella was significantly associated with hygienic practice. Poultry at farms with poor hygiene/ poor management / standard are severely affected than those with good hygiene/ sanitary/ management practices. (35.3%) higher prevalence of salmonella infection was recorded in poor housing system whereas (6.3%) lower infection was investigated in good housing sysem which was significantly associated with infection (Chi=44.13, p=0.000).

This might be due to absence of good sanitary / bedding of poultry house and feed, water contamination infected ones faeces and egg as well. This result was consistent with Deen *et al. (* 2001) who indicated, stresses due to transport, improper

feeding and poor hygiene, etc. might happen to these animals considering the prevailing socioeconomic conditions, knowledge and awareness of the people, particularly those from rural areas. Different authors (Deen *et al.,* 2001; Wray and Davies, 2000) have attributed various stress factors to be in favor of increased *Salmonella* prevalence. Besides this, the present finding supports the report of Davies and Hinton (2000) "Even though feed, sanitary is widely accepted as a source of possible contamination, the incidence of outbreaks being attributed to feed is very low". The detection were more or less in harmony with AL-Iedani *et al*. (2014) finding that 14% from cloacal swab, 37% from litter, 10% from water and 20% from ration of *Salmonella* isolate had identified. And also the level of contamination of dressed chicken meat was found to be slightly higher than the 11.5% prevalence report by Živkovic *et al.* (1997) on market ready dressed chicken meat, in Zagreb, Croatia and 4.2% by Zhao *et al.* (2001) from Greater Washington D.C. area. Variation in the frequency of isolation of *salmonella* between the present and earlier studies in Ethiopia might stem from either actual difference in prevalence of *Salmonella* in carrier chicken in the flock of origin or the fact that, unlike our studies, giblets were included in previous studies, which contributed substantially for the difference in prevalence.

Similarly, according to D'Aoust (1989) high prevalence of *Salmonella* in chicken carcass is attributable to problems associated with poultry husbandry, processing, and cross-contamination of carcasses in slaughtering plant through common scalding, de-feathering, and chilling processes. The same author also showed that cross-contamination from the hands of workers, equipment and utensils can spread the bacterium to uncontaminated carcass and parts. The relatively high prevalence of *Salmonella* in dressed chicken carcass might have emerged, in part, from their feeding habits i.e., their daily ration comprises of animal proteins, as source of essential amino acids and minerals, that might have been contaminated with *Salmonella* (D'Aoust, 1989; Pegram, 1981). Similar result was reported by Netsanet *et al*. (2012), who indicated, the low prevalence in the intensive farms might be due to a relatively good management practice including ventilation, proper spacing and relatively trained workers whereas high prevalence of infection in semi-intensive system due to economic reason to accommodate good housing with trained personnel.

The findings of (25.2%) high prevalence of salmonella in farms with floors bedding was diagnosed whereas (17.02%) lower infection was recorded in cage types, which influence the occurrence of salmonella, and was not statistically significant (p> 0.05), this result was concord with finding of Al-Abadi and Al-Mayah (2012) 19.1 % salmonella isolated from fecal samples. Comparably low result was reported by Tessema K. *et al*., (2017) in Haramaya poultry farm, 2.3% and 3.3% salmonella positive egg samples were recorded from cage and floor house system respectively; however, there was no statistically significant difference (P>0.05) in the prevalence of *Salmonella* among the two house systems. The slight increase of prevalence might be due to poor housing system which have access to entrance of carriers of salmonella like rodents, birds and pests to poultry farm and cross contamination also associated with farm workers, hygienic status, air quality, confinement of birds and dust originated from feed and faeces may contain large number of microorganisms and this poor system favor the proliferation and transmission of salmonella pathogens. It could also be due to contamination from equipment, floor and hands of personnel, as has been reported by various authors (Baird-Parker, 1990; Smeltzer *et al.,* 1980b; Smeltzer *et al*., 1980a; Smeltzer *et al.,* 1979; Watson, 1975). Comparably, Baird-Parker (1990) reported that, the main sources of infection are infected chickens transferred via environment contamination.

The prevalence of salmonella in local chicken was (18.42%) whereas infection in cross/ exotic/ breed was (26.29%) which was not significantly associated with the occurrence of salmonellosis (p>0.05, chi=3.19). This finding was lower when compared with the reports made by by Zhao *et al.* (2001) from Greater Washington D.C. area, it is of interest to note that 69.2% of dressed chicken carcass sampled, originated from indigenous backyard local chicken with different management from commercial farms. Unlike the previous studies made on chicken in Ethiopia, it is of interest to note that 144 (69.2%) of dressed chicken carcass sampled in this research work originated from indigenous backyard local chicken with different management from commercial farms. Comparably lower research was reported by Tessema K. *et al*., (2017) in Haramaya poultry farm, who indicated, the prevalence of *Salmonella* in eggs on the bases of chicken breed sources was 2.9%, 3.8% and 2% for Bovans, Fayoumi and White leg horn, respectively; the prevalence difference was not show statistical significance (P>0.05) between the rate of detecting *Salmonella* spp., and non-significant analytical situation was observed in eggs sampled from different chicken breeds. This is presumably due to unequal exposure to the risk factors as the breeds were housed in different house system. This difference might be due to Fayoumi breed was kept in the floor house system in which there is lower hygienic and high cross contamination between the flock eggs at

laying than the cage house system. Other study also reported that one day- old chicks orally infected with *S. pullorum* produced contaminated eggs frequently during the period of sexual maturity as a consequence of reproductive tract colonization (Wigley p. *et al*., 2001).

The effect of different risk factors such as sex, age categories, study sites and body conditions on prevalence of chicken salmonellosis was studied and, statistically significant associations were observed in age groups, body conditions and study sites (p<0.002) while sex groups were not found to be statistically signifcant (P>0.05). This result is in agreement with previous reports of (Wigley p. *et al*., 2001). So, (35.6%) higher salmonella infection was recorded in 1-4 month years age of chicken where as lower infection (17.03%) was diagnosed in >4month-7month years of age chickens which was statistically significant (p<0.002, Chi=12.01). And also body condition had a significant influence on the occurrence of salmonella, higher prevalence (62.68%) of salmonella infection was recorded in poor body conditions whereas 12.9% and 16.6% salmonella infection were observed in medium and good body conditions respectively, which was significantly associated with salmonella infection (chi=71.78, p<0.000). Higher prevalence of salmonella infection was recoreded in male (26.82%) where as lower infection was registered in female (20.45%) sex categories, which was not statistically significant (chi=2.14, p>0.05). The fact that salmonellosis do not depend on gender could possibly be hypothesised that both male and female animals have virtually equal chance of being in contact with infection and ultimately developing the disease.

Many reports on treatment trials of *Salmonella* infection do not contain detailed descriptions of host factors of the treated animals, or of the strains causing the infections that are treated (Barkema *et al*., 2006). In the previously infected animals, the Salmonella isolates which were responsible to the previous infection were not eliminated by the effect of various antibiotics which was related to the development of drug resistance by Salmonella organisms. But mainly, salmonellosis is a complex disease involving interactions of several factors, mainly of management, and factors relating to animal and causative organisms (Tessema K. *et al*., (2017). Previous treatment history of poultry had a significant influence on the occurrence of salmonella infection, 22.2 % of salmonella was reported in previously treated poultry where as 23.87% infection was registered in not previously treated case of salmonella, which were higher. This result was not significant (Chi=0.14, Pr >0.05). Similarly, 24.06% salmonella infection was recorded in treated poultry whereas 22.7% infection was recorded in not treated case of salmonella, which were not statisticall significant difference (Chi=0.08, Pr> 0.05). The possible fair judgment for this could be that inappropriate implementation of antibiotics to treat salmonella case in some part of the study area leading to occurrence of an isolate which had a potential of drug resistance.

5.3 Frequency of motile isolates

Salmonella in poultry are commonly classified into two groups on the basis of the diseases caused. The first group which consists of the poultry host-adapted, pathogenic, non-motile *Salmonellae*, *S. pullorum* causes pullorum disease in chickens, and *S. gallinarum* is responsible for Fowl typhoid (Kwon *et al*., 2000). The second groups of *Salmonellae* are known as the paratyphoid *Salmonellae* and, they contain the two motile leading serotypes that are responsible for human infection, *S. typhimurium,* and *S. enteritidis* (Gast, 2003). The serotypes, *S. typhimurium*, and *S. enteritidis*, which produces illness in humans, usually remain sub-clinical in layer birds (Quinn *et al*., 2002). Accordingly, most of non- host specific, motile *Salmonella* in poultry are probably zoonotic which cause disease in humans through food chains. With this view and understanding that motility tests were conducted for all 89 *Salmonella* isolates identified by culture and biochemical tests methods. Accordingly, 80 (89.8%) were non motile while 9(10.1%) were found motile. This findings was high as compare to, Jahan *et al.* (2012) in Bangladesh, and F. Abunna *et al*. (2016) in and around Modjo, (59.26%, motile v 40.74%, non-motile) and (67.74% motile v 32.3% non motile) salmonella respectively by motility test. The motile isolates were suspected to be zoonotic serovars like *S. typhimurium,* and *S. enteritidis* while non motile once suspected as poultry adapted salmonellosis (*S. pullorum* and *S. gallinarum*).

Regarding culture methods, since the isolation and correct identification of *Salmonella* are very crucial for the characterization purpose, the colonies having typical cultural characteristics were selected as presumptive for *Salmonella* serovers. In this study several selective media such as SS, EMB, XLD were used simultaneously to culture the organism because all of them are not equally suitable for all the serovars of Salmonella. In the present study, specific enriched media were used for the isolation and identification of *Salmonellae* which was also used by a number of researchers such as Hyeon JY *et al.,* (2012), Muktaruzzaman *et al.,* (2010), Habrun, and Mitak, (2003). The colony characteristics of *Salmonella spp.* found in this study was translucent, black, smooth, small round colonies on SS agar, Pink color colony on EMB agar and pink color colony with black centre in XLD agar, were similar to the findings of other authors (Muktaruzzaman *et al.,* (2010) Sujatha *et al*.,

(2003) Habrun, and Mitak., (2003). Of which 89 samples were detected as positive for *salmonella spp.*

5.4 Antimicrobial susceptibility pattern

Frequency of mono resistant isolates

Of all 46 *Salmonella* isolates screened for antimicrobial susceptibility test against ten antimicrobials. All the isolates were susceptible to Ciprofloxacilin, Gentamycin and sulphonamides. The reason why these antimicrobials were less resistant/susceptible/ might be that they are not used in the study area in veterinary clinics or services and even not frequently used (infrequent use of therapeutics) perhaps in human medicine.

This finding is similar with finding of Begum *et al*. (2010) on *Salmonella* isolates from chicken eggs, intestines and environmental samples**.** For the rest 7 different drugs, 43 (97.82%) were resistant to one or more of antimicrobials. This finding was in agreement with a numbers finding on *Salmonella* antibiogram tests, for isolates from poultry and poultry products samples like Maria (2010) from America, Jahan *et al*. (2012) in Bangladesh, Tabo *et al*. (2013) in Chad, Carraminana *et al*. (2004) from Spain. However, the current finding is not in agreement with results of Singh *et al*. (2013) from India, and Antunes *et al*. (2003) from Portugal, but different with resistant patterns. Disagreement may be due to different strains of isolates and/or difference in levels of strains' resistivity.

Accordingly, 39 (84.8%), 37 (80.43%), 35(76.08%), 32(69.56%), 31 (67.39%), 26 (56.52%), and 22 (47.82%) were resistant to Tetracycline, Streptomycin, kanamycin, Norfloxacin, Trimthoprim, Nalidixic acid and Chloramphenicol respectively. High resistant to Tetracycline, Streptomycin, Nalidixic acid, Norfloxacin, Kanamycin, Sulphamethoxasole-Trimethoprim were in agreement with what Maria, (2010) and Jahan *et al*. (2012) found on poultry related resistant isolates. And also this finding goes with what Davies (1996) found that most of the *Enterobacteriaceae* family including *Salmonella* is resistant to the drugs including Aminoglycosides, beta lactams, Trimethoprim and Chloramphenicol. Similar research was reported by Tessema K. *et al*., (2017) in Haramaya poultry farm, who indicated, 72.7% were resistant to one or more of the tested antimicrobials and the most common resistance observed was tetracycline (72.7%). However, spectinomycin, kanamycin and chloramphenicol were effective against most of the *Salmonella* isolate. Comparable result was report was by Beshatu F. (2014) in Dire Dawa municipal abattoir, who showed, highest level of resistance was observed for tetracycline (100%), nitrofurans (100%), streptomycin (81.8%) and kanamycin (79.5%).

Of 46 resistant isolates to anyone of the 10 drugs, 39 isolates were only resistant to Tetracycline while the rest isolates were resistant to at least for two of the 9 different drugs. Consequently, (39/46) isolates were resistance to Tetracycline. Thus, Tetracycline was the most common single resistance (84.8%). These may be due to wider use of Tetracycline and its affordable nature from local pharmacy and most frequently utilized and exposed antimicrobials from among all veterinary drugs in Ethiopia. Similarly, a possible development of resistance from prolonged and indiscriminate usage of some antimicrobials. Hence, tetracycline are the only most commonly used antimicrobials for the treatment of other infections as well as salmonellosis in veterinary practice in Ethiopia, as the result, there was spread of drug resistance reported by many researchers which was in line with the recent findings.

Multi-drug resistance

Out of 46 isolates, 44(95.65%) were /MDR/ resistant to different combinations of two or more tested antimicrobials and the remaining 1(2.2%) isolates were non multi drug resistance/non MDR/. Besides this, 6 (13.04%) of the isolates were the most frequent multidrug resistant pattern to four drugs which were, Tetracycline, Streptomycin, kanamycin and norfloxacin. From the total pure isolated *S. gallinarium,* 2(4.4%), 3(6.5%), 4(8.7%), 7(15.2%), 8(17.4%), 9(19.6%), and 12(26.08%) of the isolates were resistant for 2-10 drugs, respectively. Comparably result was reported by F. Abunna *et al*., (2016) in and around Modjo, Central Oromia, and Ethiopia, who indicated, 18 (94.73%) of multi-drug resistant (MDR) isolates were found resistant to five to seven different antimicrobials. The present finding was concord with the findings of Payne *et al*. (2006) on broiler farms in which 96% of the isolates were resistant to greater than one antimicrobial agent (s) and Silvia *et al*. (2005) all strains isolated from poultry related samples were resistant to at least one antimicrobial agent. All except one (45/46) multi-drug resistant isolates were resistant to two to ten (2-10 drugs) different antimicrobials. Only one isolate was resistant to two different antimicrobials, 8(17.39%) isolates are resistance to 4 drugs, 7(15.22%) isolates are resistance to 5 drugs, 4(8.69%) isolates are resistance to 7 drug, 9 (19.56%) isolates were resistance to 8 drugs, 12 isolates (26.08%) were resistance to 6-9 drugs, and 3(6.52%) isolates were resistance to 10 drugs. Eight isolates (17.4%), 7 isolates (15.2%), 4 isolates (8.7%), 9 isolates (19.6%), 12 isolates (26.08%), 3 isolates (6.5%) resistant to isolates were shows tetra-, penta-, hepta, octa, hexa v nano, and deca respectively, with different resistance patterns. This result was similar with the findings of F. Abunna *et al.* (2016) in and around modjo, reported, 2,

5, 4, and 7 isolates were tetra-, penta-, hexa-, and hepta resistant, respectively.

This finding support the one that Sangeeta *et al.* (2010) reported on resistant isolated from chicken eggs poultry farms and from markets in that two isolates were resistant to as many as 10 antibiotics whereas, 2 isolates were resistant to 9 antibiotics, 2 to 8 and 5 to 7 antibiotics. It also seems consort with that of Jahan *et al*. (2012) in which out of 27 multi-resistant isolates, five isolates were resist to five different antimicrobials, 6 to 8, 7 to7, and 7 to 8 different antimicrobials with different resistance patterns. These all multidrug *Salmonella* isolates were confirms what Poppe *et al*. (1995 and 2002) reported as saying *Salmonella*e are among those most known to carry plasmids, which encode for drug resistance R (resistance) plasmids. This implies that widespread use of antimicrobials in animals or humans may cause an increase in the frequency of occurrence of bacteria resistant to other antimicrobials as the R plasmid may encode resistance to additional antimicrobials.

Antimicrobial resistant *Salmonella* isolates to commonly used antimicrobials were detected; all isolates were resistant at least for one antimicrobial. However, all the isolates were susceptible to, ciprofloxacin, gentamycin and sulphonamides. All of the total isolates were resistant to one or more of the tested antimicrobials; 95.6 % were multiple antimicrobial resistant while the rest 2.2 % were resistant to single antimicrobial. This finding is in contrast to Zewdu (2004) who reported 25% antimicrobial resis-tant *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 (D'Aoust, 1997).

The effective-ness of gentamycin, ciprofloxacin, and sulphonamides in 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 bacte-ria. Different individuals reported antimicrobial resistant *Salmonella* isolates in previous studies from Ethiopia (Gedebou and Tassew, 1981; Ashenafi and Gedebou, 1985; Molla *et al*., 1999; Molla *et al*., 2003) and from other countries (D'Aoust *et al*., 1992; White *et al*., 2001). The findings of 100% antimicrobial resistant *Salmonella* isolates from examined dairy items samples were remar-kable. It represents public health hazard due to the fact that food poisoning outbreaks would be difficult to treat and this pool of MDR *Salmonella* in food supply repre-sents a reservoir for the transferable resistant genes (Diaze De Aguayo *et al*., 1992). This multi drug resistance occurred might be due to administration of multiple antibiotics for prophylaxis or infection, lack of drug sensitivity tests in the poultry farms, uncontrolled or discriminate use of antibiotics in the farms and another possibility is that poultry/ chickens/ are being treated with antibiotics for other conditions, thereby selecting for resistant populations of *S. gallinarium* (Shitandi and Sternesjo, 2004). Similarly, comparable result was reported by Iwabuchi *et al.,* (2011) described that among 452 *Salmonella* isolates, 443 (98.0%) were resistant to one or more antibiotics, and 221 (48.9%) showed multiple antibiotic resistance, thereby implying that multiple-antibiotic resistant salmonella organisms are widespread in chicken meat in Japan and resistance to oxytetracycline was most common (72.6%), followed by dihydrostreptomycin (69.2%).

Most isolates showed high level of susceptibility to Ciprofloxacin which is in agreement with Harsha *et al*. (2011) who described Ciprofloxacin as an increasingly demanded and successfully used to treat septicemic case in humans and *Salmonella* isolates resistance to Ciprofloxacin has been found occasionally. The antimicrobial susceptibility test result revealed that the isolated bacterium that were subjected to ten different antibiotics found only non-resistant to ciprofloacin. Bacteria having MAR Index > 0.2 originate from an environment where several antibiotics are used (Tambekar *et al*., 2006).

4. Conculusion And Recommendations

Salmonellae are an important group of pathogens responsible for human and animal diseases.

Among the 89 (23.2%) positive isolates, 7 fermented glucose and maltose and produced both acid and gas and did not ferment dulcitol which is positive for *S. pullorum*. 80(89.88%) of the positive isolates fermented glucose, maltose and dulcitol and produced acid, which are typical for *S. gallinarum*, the remaining two were *S. typhimurium.* So, identified Salmonella spp were isolated *by* bacteriological cultural and biochemical methods. Origin/sites/, age categories, body condition, and sanitary/management condition were potential risk factors, which were statistically significant value for salmonella infection ($p<0.000$) whereas sex groups, floor type, previous treatment history and breed factors were not significant ($p>0.05$). Of the 89 isolates, 2.24 % were motile (contributes to zoonoses) while (97.75%) were non-motile. Almost all isolates were resistant to one or more of the tested antimicrobials. Of all isolates, 95.6 % were multidrug resistant (MDR). 84.78%, 80.43%, 76.08%, 69.56%, 67.39%, 56.52% and 47.82% of the isolates were resistant to Tetracycline, Streptomycin, Kanamycine, Norfloxacin, Trimthoprim, Nalidixic acid and Chloramphenicol respectively. However, the majority of the isolates were susceptible/ less

resistance/ to ciprofloxacin and gentamycin, followed by sulphonamides.

Based on the above conclusion, the following recommendations are forwarded:-

- poultry farms are a potential source of *Salmonella* infection with antimicrobial resistance, and significant threat to public health particularly to those who have direct or indirect contact to poultry and poultry products so, hygienic management of poultry products and prudent use of antimicrobials are also suggested.
- Identified potential risk factors should be managed properly inorder to minimize the transmission of salmonella species.
- Biosecurity measures should be strictly applied in poultry farms where cross contamination was high.
- Chickens should be checked for healthyness and adaptation of the enviroment for that particular area before rearing was planned to design and also precondition, predisposing factors should be assessed before production was conducted in farms so as to reduce or eradicated salmonellosis which was carrier once infect the chickens.
- Since *Salmonella* is resistant to most common drugs, attention should be taken in selecting antimicrobials in treating *Salmonella* infection both in animals and human being based on antimicrobial sensitivity test.
- Further study, on molecular characterization for serotyping and *salmonella* population structure genetic studies along with genes responsible for pathogenecity and drug resistance of the isolates of *Salmonella*.

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