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**Review Article**

# Antimicrobial Resistance Patterns of *Salmonella* in Ethiopia Since 2009/2010: A Review

**Samuel Chane Teferi**

College of Natural and Computational Sciences, Department of Biology, Salale University, Fitche, Ethiopia

**Email address:**
Samuelchane19@gmail.com

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**Abstract:** *Salmonella* is one of the major and important foodborne pathogens of humans and animals causing salmonellosis, which have great medical and economical cost. Infections with *Salmonella* in food-producing animals present a serious public health concern, because food products of animal origin are considered to be a significant source of human infection. Most common sources of infection are eggs and related products, and meat from poultry and other food animal species. Milk and dairy products have also been associated with outbreaks of salmonellosis in people. Studies indicated the widespread occurrence of antimicrobial resistance in Ethiopia. The emergence and persistence of antimicrobial resistance is driven by varied factors including the indiscriminate use of antibiotics and variable drug efficacy and presents a major threat to the control of infectious diseases. In recent years, since the rate at which resistance occurs has outpaced the development of new drug replacements, it has become necessary to use the currently available agents, optimally and appropriately. Therefore, developing strategies in order to minimize the expansion of antimicrobial resistance is critically important. Performing drug susceptibility test for each salmonellosis case and Educating the community about *Salmonella* transmission is very important. Moreover, collaboration of different stakeholders like the public health, animal health, and animal agriculture communities plays a pivotal role.

**Keywords:** *Salmonella*, Ethiopia, Antimicrobial Resistance, Public Health

## 1. Introduction

Non-typhoid *Salmonella* are re-emerging as one of the most important etiological agents of infectious diseases in the world. Multi-antibiotic resistance in non-typhoid *Salmonella* has been associated with enhanced virulence and excess mortality in patients compared with infection with sensitive strains. High rates of resistance to multiple antimicrobial agents (resistance to three or more classes of antibiotics) by enteric pathogenic were previously reported from Libya [54].

Infections with *Salmonella* in food-producing animals present a serious public health concern, because food products of animal origin are considered to be a significant source of human infection. Most common sources of infection are eggs and related products, and meat from poultry and other food animal species. Milk and dairy products have also been associated with outbreaks of salmonellosis in people. In addition, contamination of fruit and vegetables by infected water may also be a source of infection [35].

Gastroenteritis is the most common *Salmonella* infection worldwide, accounting for 93.8 million cases which result in 155,000 deaths per year [42]. In spite of improvements in hygiene and sanitation, the incidence of NTS infections continues to increase, creating a burden in both industrialized and underdeveloped countries [42].

*Salmonella* spp. are Gram-negative, non-spore forming rod-shaped bacteria and are members of the family *Enterobacteriaceae* [36]. The genus *Salmonella* is divided into two species: *S. enterica* (comprising six subspecies) and *S. bongori.* Over 99% of human *Salmonella* spp. infections are caused by *S. enterica* subsp. [16].

Amongst *Salmonella* species, antimicrobial resistance is a well confirmed phenomenon and antimicrobial-resistant *Salmonella* are increasingly associated with the use of antimicrobial agents. However, the excess or overuse of antimicrobials can generate genomic selective pressures to enable microbes to adapt and acquire resistance [72, 30].

Ultimately, increases in bacterial antimicrobial resistance pose a considerable threat to public health, especially for vulnerable populations, including young children [57], the elderly and immunocompromised individuals [34].

International trading and its introduction through international travel, human migration, food, animal feed and livestock trade are also other challenges; Water source: *Salmonellae* can be found in contaminated water; Inanimate objects. Moreover, in recent years, antimicrobial resistance of *Salmonella* has increased worldwide, due to the widespread use of antimicrobial drugs in the human and veterinary sectors, is the other ambiguities in the food processing environment [50]. Studies conducted in Ethiopia on salmonellosis which suggests an increase in the antimicrobial resistance of *Salmonella* to commonly used antimicrobials in both public health and veterinary sectors [25, 23, 71, 5, 3, 10, 58, 41, 2].

Acquisition of new genetic material by antimicrobial-susceptible bacteria from resistant strains of bacteria may occur through conjugation, transformation, or transduction, with transposons often facilitating the incorporation of the multiple resistance genes into the host's genome or plasmids [19, 60]. Information on the antimicrobial resistance pattern of the *Salmonella* isolates is useful for successful treatment and also used for planning to minimize resistance in the future. But in Ethiopia surveillance and monitoring systems are not good enough and antimicrobial profile of the isolates has not been sufficiently studied and even the studies done in different part of Ethiopia is not compiled. So, the objective of this paper is to review the prevalence and antimicrobial resistance patterns of *Salmonella* isolates conducted in Ethiopia since 2009/10.

# 2. Overview of Salmonellosis

The genus *Salmonella* obtained its name from the American veterinarian Daniel Elmer Salmon, who first isolated *Salmonella enterica* serotype Choleraesuis from pigs in 1885 [53]. In recent years, the issue of nomenclature of the genus Salmonella has been complex, controversial, and still remains subject of debate [24]. At present, most *Salmonella* reference centers in the world including the Centers for Disease Control (CDC) adopt the nomenclatural system of *Salmonella* as recommended by the World Health Organization (WHO) [52]. This nomenclatural system classifies the genus *Salmonella* into two species based on differences in their 16S rRNA sequence analysis. These two broad species included S. enterica (type species) and S. bongori [24].

## *2.1. Salmonellosis and Pathogenesis*

Once ingested, *Salmonella* spp. must survive the low pH of the stomach, adhere to the small intestine epithelial cells and overcome host defense mechanisms to enable infection [36]. *Salmonella* spp. possesses a number of structural and physiological virulence factors, enabling them to cause acute and chronic disease in humans. The virulence of *Salmonella* spp. varies with the length and structure of the O side chains of lipopolysaccharide molecules at the surface of the bacterial cell. Resistance of *Salmonella* spp. to the lytic action of complement (part of the immune response) is directly related to the length of the O side chain [36]. Other important virulence factors include the presence and type of fimbriae, which is related to the ability of *Salmonella* spp. to attach to host epithelium cells, as well as the expression of genes responsible for invasion into cells [38]. Some of these virulence genes are encoded on *Salmonella* pathogenicity islands (SPI). SPI-1 is required for bacterial invasion into intestinal epithelial cells, while systemic infections and intracellular accumulation of *Salmonella* spp. are dependent on the function of SPI-2 [64].

*Salmonella* spp. produces a heat labile enterotoxin, resulting in the loss of intestinal fluids (causing diarrhoea). This enterotoxin is closely related functionally, immunologically and genetically to the toxin of *Vibrio cholerae* and the heat labile toxin of pathogenic *Escherichia coli* [36]. Most *Salmonella* strains also produce heat labile cytotoxin which may cause damage to the intestinal mucosal surface and results in general enteric symptoms and inflammation. Infection with non-typhoidal *Salmonella* is generally limited to a localized intestinal event. However, the presence of virulence plasmids has been associated with non-typhoidal *Salmonella* spp. surviving in phagocytes and spreading from the small intestine to the spleen and liver [36].

## *2.2. Salmonellosis in Man*

The broad host-range *Salmonella* serovars are prevalent within warm-blooded animal populations that make up the human food supply and bacterial transmission generally results from consumption of raw or undercooked food products [38].

Human salmonellosis is usually characterized by acute onset of fever, abdominal pain, diarrhea, nausea and sometimes vomiting [69]. Typically, symptoms of gastroenteritis develop within 6 to 72 hour after ingestion of the bacteria. The symptoms are usually self-limiting and typically resolve within 2 to 7 days. In a small percentage of cases, septicemia and invasive infections of organs and tissues can occur, leading to diseases such as osteomyelitis, pneumonia, and meningitis [13]. In some cases, particularly in the very young and in the elderly, the associated dehydration can become severe and life threatening. In such cases, as well as in cases where *Salmonella* causes bloodstream infection, effective antimicrobials are essential drugs for treatment. Serious complications occur in a small proportion of cases [69].

With respect to human disease, *Salmonella* serotypes can be divided into three groups that cause distinctive clinical syndromes, typhoid fever, bacteremia and enteritis [56]. The non-typhoid *Salmonella* serotypes can cause protean manifestations in humans, including acute gastroenteritis, bacteremia and extra intestinal localized infections involving many organs [14]. Within *Salmonella* enterica subspecies I (S. enterica subspecies enterica), the most common O-antigen serogroups are A, B, C1, C2, D, and E. Strains within these serogroups cause approximately 99% of *Salmonella* infections in humans and warm blooded animals. Serotypes in other subspecies are usually isolated from cold-blooded animals and the environment, but rarely from humans [66].

### *2.3. Salmonellosis in Animals*

*Salmonella* serotypes have a broad host range [56] and prevalent in the warm blooded animal population including rodents. Reptiles kept as pets, such as turtles, iguanas, other lizards and snakes, are often identified as non-food sources of infection. Some serotypes are highly adapted to animal hosts, such as *Salmonella gallinarum* in poultry and *Salmonella abortusovis* in sheep. Many non-typhoidal *Salmonella* strains, such as *Salmonella typhimurium* and *Salmonella enteritidis*, infect a wide range of animal host including poultry, cattle and [51]. These serotypes generally cause self-limiting gastrointestinal infections usually less severe than enteric fever in humans. However, they also have the capacity to produce typhoid-like infections in mice and in humans or asymptomatic intestinal colonization in chickens [66].

*Salmonellosis in food animals in Ethiopia*

*Salmonella* are widely distributed in nature and they survive well in a variety of foods. Poultry, eggs and dairy products are the most common vehicles of salmonellosis. In recent years, fresh produce like fruits and vegetables have gained concern as vehicles of transmission where contamination can occur at multiple steps along the food chain [12].

*Salmonella* serotype and antimicrobial resistance surveillance and monitoring system in Ethiopia is not as organized as in developed countries. A research conducted by [42] on chicken and different chicken products in Ethiopia indicated the presence of different serotypes of *Salmonella*. The study isolates 80 *Salmonella*, 8 different serotypes were identified of which *Salmonella braenderup, S. typhimurium var. copenhagen*, *Salmonella anatum*, *Salmonella kottbus* and *Salmonella typhimurium*. According to [74]: serotypes isolated include *Salmonella bovismorbificans*, *Salmonella hadar* and *Salmonella infantis*. *S. braenderup*, *S. anatum* and *Salmonella newport* appear to be the major *Salmonella* serotypes associated with chicken meat and chicken meat products around Addis Ababa.

## 3. Health Impact of Salmonellosis

The incidence of non-typhoidal salmonellosis has doubled in the United States over the past two decades. The center for disease control estimates that there are 2 million cases annually, with 500 to 2000 deaths [13]. Although more than 200 serovars of *Salmonella* are considered to be human pathogens, the majority of the reported cases in the United States are caused by *S*. Typhimurium or *S*. Enteritidis [26]. In most parts of the world, countries have seen dramatic and continuous increases in human outbreaks of salmonellosis, caused by infections in animals. In 2004, in the European Union (EU) alone, 192,703 human cases of salmonellosis were reported. These and similar data from other countries almost certainly underestimate the magnitude of the problem, as many cases of salmonellosis are not reported. In addition to human health implications, it also generates negative economic impacts due to surveillance investigation, and illness treatment and prevention [33]. Financial costs are not only associated with investigation, treatment and prevention of human illness, fall in to the public and privet sectors and may be surprising, both in terms of the levels of costs incurred and the variety of affected. In the public sector, resources may be diverted from preventive activities in to the treatment of patients and investigation of the source of infection. Cost estimates per case of human salmonellosis range from approximately US $40 for uncomplicated cases to US$ 4.6 million for cases ending with hospitalizations and deaths [69]. The costs of food-borne salmonellosis alone are estimated to reach up to € 2.8 billion annually in EU countries altogether. In Denmark, the annual estimated cost of food-borne salmonellosis is US$ 15.5 million, representing approximately 0.009% of salmonellosis in the country. A *Salmonella* control program has been conducted for several years in the country, and the annual cost of this control program is estimated around US$ 14.1 million [69].

In the Netherlands, annual costs caused by human salmonellosis are estimated between 32 and 90 million Euro [65]. Although few developed countries have managed to report data on the economic cost of *Salmonella,* data related to the cost of foodborne disease are generally not available from developing countries [69].

## 4. Antimicrobial resistance of *Salmonella*

### *4.1. Global Trends in Antimicrobial Resistance Patterns*

Feeds have been responsible for the infection of poultry with multidrug-resistant Nontyphoid *Salmonella* in several industrialized countries. In food animal production, antimicrobials are administered for therapeutic means, for treatment of infection, prophylactic and nontherapeutic purposes for growth promotion and improved feed efficiency [68]. The usage of growth promoting agents (GPAs) in food animal production is a major public health threat because this practice can contribute to the emergence of antimicrobial resistance worldwide [39, 59, 67].

The resistance towards the traditional first-line antibiotics such as ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole define multidrug resistance (MDR) in *Salmonella* enterica [17]. Due to the use of antibiotics for the promotion of growth and prevention of disease in food animals, there is an increase of human salmonellosis cases caused by foodborne MDR *Salmonella* nowadays [72].

### *4.2. Resistance Pattern in Ethiopia*

Antimicrobial resistance is a global problem in general, but it might be more severe in Ethiopia where there is lack of antimicrobial resistance assessments of *Salmonella* and lack of rigorous regulations, but there is easy access of antimicrobials for purchase of people without prescription and incomplete treatment courses as the result of patient non-compliance [10].

There have been studies conducted in Ethiopia on salmonellosis (Tables 1 and 2) which suggest an increase in the antimicrobial resistance of *Salmonella* to commonly used antimicrobials in both public health and veterinary sectors [25, 23, 71, 5, 3, 10, 58, 41, 2, 27, 29, 61].

According to [10] multiple drug resistant *Salmonella* organisms detected in their study on aetiology of febrile and diarrheic illness in Ethiopian children focusing on *Salmonella*. In a study conducted by [70] and [55], 100% of the isolates had varying resistance to the tested antibiotics. Multiple drug resistance was observed in all 100% of the *Salmonella* isolates. High proportion of *Salmonella* isolates developed resistance to the commonly prescribed antimicrobials and this may be a considerable risk in the treatment of clinical cases [4]. In addition, according to [58] out of the 87 isolates, 18 (20.7%) *Salmonella* serovars consisting of Newport (n = 14), Anatum (n = 3) and Eastbourne (n = 1) were resistant to two or more antimicrobials. Among the antimicrobial resistant *Salmonella* serovars, S. Newport was multidrug resistant (15.6%) and exhibited resistance to streptomycin, sulphisoxazole and tetracycline. According to [11] out of the 42 isolate, 14 different *Salmonella* serotypes were recovered, the predominant serotypes were S. Bronx (n = 7; 16.7%), S. Newport (n = 6; 14.3%), S. Typhimurium (n = 4; 9.5%), S. Indiana (n = 4; 9.5%), S. Kentucky (n = 4; 9.5%), S. Saintpaul (n = 4; 9.5%) and S. Virchow (n = 4; 9.5%). Other serotypes such as S. Anatum (n = 2), S. Haifa (n = 2), S. Braenderup (n = 1), S. Chailey (n = 1), S. Minnesota (n = 1), S. Muenchen (n = 1) and S. Tarshyne (n = 1) were also identified. Different serotypes appeared to exhibit disparity in their susceptibility to some of the antimicrobials tested. For instance, all S. Newport isolates were resistant to three or more antimicrobials. Likewise, 3 of the 4 S. Saintpaul isolates were resistant to five or more antimicrobials. On the other hand, strains belonging to S. Virchow, S. Typhimurium and S. Kentucky were resistant to relatively less number of antimicrobials

# 5. Control and Prevention of Salmonellosis

Prevention of salmonellosis by the implementation of hygiene measures is difficult and use of antibiotics may give rise to the emergence of resistance problems [45]. Additional measures to control secondary contamination could be prevention of contamination by cleaning and disinfection, hygiene of personnel and proper processing.

Growth of micro-organisms in meat and poultry products can be controlled by maintaining a cold chain at 10°C, especially for *Salmonella* during transport and storage [15]. The use of program aimed at the prevention and control of *Salmonella* and other zoonotic bacteria in primary animal production, can lead to a reduction in the level of contamination of related food products at retail, and thereby also reduce the risk of human exposure to antimicrobial resistant *Salmonella* from those food products. The occurrence of *Salmonella* and antimicrobial resistant *Salmonella* in other food commodities is also likely to be reduced as the risk of cross-contamination is reduced [22].

***Table 1.*** *Antimicrobial resistance profiles of Salmonella isolates in animals, Ethiopia.*

| Year | Location | Species | No. of sample | No. isolates Tested | MDR No. (%) | Predominant serovars Isolated No. | Common resistance pattern | Maximum drug resisted No. | References |
|---|---|---|---|---|---|---|---|---|---|
| 2009 | Addis Ababa | Poultry, Cattle | 730 | 51 | 15 (29.4) | - | AMX, AMP, CIP, GEN, KAN | 7 | [9] |
| 2009 | Debre Zeit | Cattle | 800 | 87 | - | Anatum (54), Newport (18) | S, SXT, TET | 3 | [58] |
| 2009/10 | Jimma | Cattle | 180 | 8 | 8 (100) | - | AMP, CHL, NAL, S, TET | 5 | [7] |
| 2010 | Addis Ababa | Cattle | 195 | 21 | 10 (47.6) | - | AMP, S, ET, CF | 8 | [3] |
| 2011 | Bahir Dar | Cattle | 186 | 28 | 4 (14.3) | Typhimurium (6), Newport (6) | AMP, GEN, NOR, S, TET, TMP, CHL | 8 | [47] |
| 2011 | Addis Ababa | Dairy items | 384 | 6 | 3 (50) | - | TET, AMP, AMX, CHL | 8 | [41] |
| 2012/13 | Haramaya | Chickens | 300 | 8 | 8 (100) | - | CLN, ERY, AMP, AMX, TET | 10 | [37] |
| 2013 | Tigray | Cattle origin food | 384 | 63 | 45 (71.4) | Typhimurium (40), Enteritidis (33) | CF, CHL, TET, GEN, SUV, SXT, KAN, S, NEO | 12 | [2] |
| 2013/14 | Debre Zeit and Modjo | Exotic Chicken | 384 | 56 | 43 (86) | - | AMP, SXT, OXT, NAL, CHL | 9 | [20] |
| 2014 | Holeta | Abattoir and dairy farm | 232 | 13 | 9 (69.23) | - | S, CHL, AMP | 9 | [27] |
| 2014/15 | SNNPR | Chickens | 270 | 45 | 45 (100) | - | K, SXT, AMP, FOX, NAL, S, TET, CHL, CIP | 9 | [55] |
| 2014/15 | Addis Ababa | Cattle | 726 | 27 | 27 (100) | *S*. Dublin 10 (35.7%) and *S*. Virchow 5 (17.86%) | S, CEP, AMP, AMX | 15 | [40] |
| 2014/15 | Gondar | Food item animal origin | 384 | 21 | 10 (47.6) | - | TET, SXT, F, NAL, GEN, CF, AMP, AMX | 8 | [46] |
| 2015 | Addis Ababa | Dog | 360 | 42 | 30 (71.4) | S. Bronx (n = 7; 16.7%), S. | OXT, NEO, S, CF, DOX, AMP, AMX | 14 | [11] |

| Year | Location | Species | No. of sample | No. isolates Tested | MDR No. (%) | Predominant serovars Isolated No. | Common resistance pattern | Maximum drug resisted No. | References |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Newport (n = 6; 14.3%) | | | |
| 2015/16 | Wolaita Sodo | Beef | 448 | 56 | 56 (100) | - | TET, F, S, KAN, AMP | 12 | [70] |
| 2016 | Meki | Cattle | 304 | 34 | 33 (97.05) | - | AMP, AMOX, S, NAL | 9 | [28] |
| 2016/17 | West Shoa (Ambo, Bako and Gojo) | Dog | 438 | 48 | 15 (31.25) | - | S, AMP, PEN, CHL, PB | 10 | [23] |
| 2017/18 | Hawassa | lactating dairy cows | 216 | 21 | 20 (96.4) | - | KAN, NAL, OXT | 9 | [25] |

AMP: Ampicillin; AMX-CAL: amoxicillin-clavulinic acid; CHL: chloramphinicol; CF: cephalothin; CIP: ciprofloxacin; GEN: gentamycin; PB: lincomycin; KAN: kanamycin; NAL: nalidixic acid; FOX: cefoxitin; NOR: norfoxacillin; DOX: doxycycline; CLN: clindamycin; STR: streptomycin; SXT: trimethoprim-sulfamethoxazole; TET: tetracycline; PEN: penicillin; TMP: trimethoprim; OXT: Oxytetracycline; NEO: neomycin; F: nitrofurantoin; S: streptomycin; SUV: sulphisoxazole; MDR: multiple drug resistance.

***Table 2.*** *Antimicrobial resistance profiles of Salmonella isolates in human, Ethiopia.*

| Year | Location | No. of sample | No. of isolates tested | MDR No. (%) | Predominant serovars isolated (No.) | Common resistance pattern | Max. Antibiotics resisted | References |
|---|---|---|---|---|---|---|---|---|
| 2009 | Bahir Dar | 384 | 6 | 6 (100) | Typhi | AMP, COT, TET | 6 | [8] |
| 2009/2010 | Jimma | 260 | 1 | 1 (100) | - | AMX, AMP, CHL, CF, TET, SXT | 6 | [63] |
| 2011 | Hawassa | 158 | 4 | 4 (100) | Serogroup B (3, 1.9%), serogroup A (1, 0.6%) | ERY, FOX, NAL | 3 | [49] |
| 2011/2012 | Bahir Dar | 422 | 33 | 30 (90.9) | *Salmonella enterica* subspecies *arizonae* | AMP, AUG, SXT, TET, CHL | 9 | [29] |
| 2011/2012 | Butajira | 382 | 40 | 14 (35) | Serogroup (typable) (15.0%) | TET, AMP, COT | 7 | [30] |
| 2011/2012 | Bahir Dar | 422 | 33 | 8 (90.9) | - | AMP, AMX | 7 | [44] |
| 2012 | Jimma | 260 | 16 | 10 (62.5) | - | AMX, S | 5 | [31] |
| 2012/2013 | Addis Ababa | 253 | 10 | - | - | NAL, CHL, SXT, AMP, AUG | 5 | [73] |
| 2013 | Gondar | 300 | 4 | 4 (100) | Typhi | ERY, FOX, NAL | 3 | [48] |
| 2013 | Addis Ababa | 382 | 40 | 1 (27.5) | Serogroup A (6) Serogroup b (5) | TET, COT, AMP | - | [30] |
| 2013 | Addis Ababa | 172 | 6 | 6 (100) | - | AMP, CLN, AMX, ERY | 6 | [3] |
| 2013/2014 | Addis Ababa | 957 | 59 | 27 (40.3) | S. Typhimurium (22, 37.3%) S. Virchow (20, 33.9%) | S, F, SUV, KAN, CF, AMP | 18 | [62] |
| 2014 | Harar | 384 | 56 | - | - | CIP, NAL, AMP, TET, SMX, CHL | 6 | [21] |
| 2014 | Gondar | 372 | 4 | 4 (100) | - | TET, AMX, AMP | 8 | [62] |
| 2016 | Jimma | 172 | 19 | 17 (89.47) | - | AMP, TET, NAL | 6 | [61] |
| 2015/2016 | Debre Markos | 220 | 8 | - | - | AMP, TET, COT | 4 | [1] |
| 2016 | Robe/ Goba | 422 | 29 | 23 (79.31) | - | AMX, TET, CHL | 3 | [5] |
| 2017 | Dire Dawa | 218 | 13 | 9 (47.4) | - | AMX, AMP, TET | 9 | [32] |
| 2017 | Adama | 204 | 2 | 1 (50) | - | AMP, GENT | 3 | [71] |
| 2018/2019 | SNNPR | 263 | 1 | - | Typhi | AMP, TET | 10 | [43] |

AMP: Ampicillin; AMX-CAL: amoxicillin-clavulinic acid; CHL: chloramphinicol; CF: cephalothin; CIP: ciprofloxacin; GEN: gentamycin; SUV: sulfisoxazole; KAN: kanamycin; NAL: nalidixic acid; F: nitrofurantoin; AUG: augumentin; SMX: sulphamethoxazole; CLN: clindamycin; S: streptomycin; SXT: trimethoprim-sulfamethoxazole; TET: tetracycline; ERY: erythromycin; FOX: cefoxitin; COT: cotrimoxazole; MDR: multiple drug resistance.

# 6. Conclusion and Recommendations

The reviewed studies indicated that many of the isolates were resistant to one or more antibiotics. Therefore, developing strategies in order to minimize the expansion of antimicrobial resistance is critically important. Performing drug susceptibility test for each salmonellosis case and Educating the community about *Salmonella* transmission is very important. Moreover, collaboration of different stakeholders like the public health, animal health, and animal agriculture communities plays a pivotal role.

# Data Availability

All the data used for this study was included and no other data available.

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