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Phenotypic and genotypic characterization of temporally related nontyphoidal *Salmonella* strains isolated from humans and food animals in central Ethiopia

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Abstract

Salmonella is one of the common causes of food-borne bacterial illnesses. The primary sources of human nontyphoidal *Salmonella* (NTS) infection are food animals. This study characterized temporally and spatially related *Salmonella* isolated during April 2013 to March 2014 from faeces of diarrhoeic human patients in Addis Ababa (n = 68) and food animals (n = 84) in Addis Ababa and surrounding districts (dairy cattle, n = 30; slaughtered cattle, n = 20; poultry, n = 26; swine n = 8). Isolates were serotyped, page typed and tested for antimicrobial susceptibility using Kirby–Bauer disc diffusion method, and genotyped by pulsed-field gel electrophoresis (PFGE). The dominant *Salmonella* serovars isolated from food animals were *S*. Saintpaul (38.1%), *S*. Typhimurium (17.9%) and *S*. Kentucky (9.5%), whereas in humans, *S*. Typhimurium (39.7%), *S*. Virchow (30.9%) and *S*. Kottbus (10.3%) were frequently isolated. Resistance to streptomycin, sulfisoxazole, tetracycline, ampicillin and cephalothin was higher in animal isolates than human isolates, and mean number of antimicrobials to which isolates were resistant was significantly higher in isolates from cattle and poultry compared to those from humans ($p < 0.05$). All *S*. Kentucky isolated from animals and humans were multidrug resistant (MDR) with shared resistance phenotype (AmpCfCipTeSuSNa). Although this study involved small sample size and was not able to show clear epidemiological linkage among isolates from various sources, genotyping by PFGE analysis demonstrated circulation of closely related genotypes of *S*. Virchow, *S*. Typhimurium and *S*. Kentucky among humans and food animals. Detection of related *Salmonella* isolates from humans and animals, the high MDR status of isolates from animals and close proximity of farms and human residential areas in the absence of appropriate biosecurity present major public health problem. Integrated surveillance of *Salmonella* serovars in humans and animals and implementation of appropriate hazard analysis and pathogen control strategies along critical points of the food chain from farm to table is recommended.

KEYWORDS
genotyping, nontyphoidal *Salmonella*, resistance, serovar

1 | INTRODUCTION

Nontyphoidal *Salmonella* (NTS) are enteropathogenic bacteria capable of causing disease in a wide range of animals and humans. Worldwide, NTS are a leading bacterial cause of acute gastroenteritis causing an estimated 93.8 million cases and 155,000 deaths annually (Majowicz et al., 2010). The primary sources of human *Salmonella* infection are food animals such as cattle, poultry and swine, mainly via contamination of carcass with the gastrointestinal content during slaughtering (Kagambèga et al., 2013; Stopforth, Lopes, Shultz, Miksch, & Samadpour, 2006). The sources and transmission routes of *Salmonella* in developing countries are poorly understood due to lack of coordinated national epidemiological surveillance systems (Kagambèga et al., 2013; Kariuki et al., 2006). As a result, the dominant serovars affecting humans and the relative contribution of different food animals as a source of *Salmonella* infection to humans are not clearly understood.

The development and increase in resistance to antimicrobials in food-borne pathogens are a major threat to public health globally. Antimicrobial-resistant microorganisms or antimicrobial resistance genetic materials originating from food animals can reach humans through the environment, food products and through direct contact with animals (Founou, Founou, & Essack, 2016). Resistance acquired by microorganisms in food animals can directly be a threat to human health in case of zoonotic pathogens such as *Salmonella* or resistance genetic determinants can be horizontally transferred from commensal microorganisms to human pathogens in the gastrointestinal tract. A study in Europe revealed strong correlation of occurrence of antimicrobial resistance in *Escherichia coli* isolated from food animals and humans (Fey et al., 2000; Vieira et al., 2011). Fluoroquinolone-resistant strains of *S*. Typhimurium and *S*. Choleraesuis originating from pigs were also reported to disseminate to humans in Taiwan (Hsueh et al., 2004). The level of antimicrobial use in a population of food animals has also been shown to be correlated with the rate of occurrence of antimicrobial-resistant microbes in humans (Aarestrup, 2005; Chantziaras, Boyen, Callens, & Dewulf, 2014) as well as rate of occurrence of antimicrobial resistance to commensal *E. coli* in animals (Chantziaras et al., 2014).

The global antimicrobial consumption in livestock in 2010 was estimated to be 63,151 tons and is proposed to rise by 67% in 2030. Ethiopia was among the group of countries estimated to use 6–7 mg/km^2 which was the fourth of the ten highest categories of antimicrobial consumption levels, although the authors have acknowledged high uncertainty in their model prediction for antimicrobial consumption for Ethiopia (Van Boeckel et al., 2015). Although accurate data on the level of antimicrobial consumption in food animals are not available in Ethiopia, several reports have shown occurrence of MDR strains of NTS from various food animals and the food animal products (Alemu & Zewde, 2012; Eguale et al., 2014; Molla et al., 2006).

Several serovars of *Salmonella* have been reported in various food animals, food products and humans in Ethiopia. In these isolates, a high level of antimicrobial resistance has been reported (Alemayehu, Molla, & Muckle, 2003; Beyene et al., 2011; Molla et al., 2006). However, little is known on the phenotypic and genotypic relatedness of *Salmonella* isolates from humans and animals. Characterization of temporally and spatially related *Salmonella* serovars from humans and food animals using phenotypic and genotypic techniques could give important information on the source of dominant serovars causing human salmonellosis and could also provide information on the common antimicrobial resistance phenotypes shared among *Salmonella* isolates from humans and animals. This study therefore aimed to characterize *Salmonella* isolates from food animals (dairy cattle, slaughtered cattle, poultry and swine) in Addis Ababa and nearby towns and those isolated from diarrhoeic clinical human patients in Addis Ababa collected at the same time. Isolates were serotyped, phage typed and screened for antimicrobial susceptibility, and selected representative isolates were also genotyped using pulsed-field gel electrophoresis (PFGE).

Impacts

- *Salmonella* Typhimurium, *S*. Virchow and *S*. Kentucky were the dominant *Salmonella* serovars isolated from food animals and clinical diarrhoeic human patients.
- There was variation in the rate of resistance to some antimicrobials among *Salmonella* isolates from various sources, and the level of MDR was significantly higher in *Salmonella* isolates obtained from slaughtered cattle, dairy cattle and poultry compared to those obtained from humans.
- Detection of closely related *Salmonella* serovars resistant to several antimicrobials from humans and food animals presents major public health problem.

2 | MATERIALS AND METHODS

2.1 | Bacterial isolates

Salmonella isolates obtained from different sources during April 2013 to March 2014 were used in this study. These isolates were collected from stool samples of diarrhoeic human patients (n = 68) in Addis Ababa (Eguale et al., 2015) and faeces of dairy cattle (n = 30) in and around Addis Ababa. Majority of these *Salmonella* isolates (n = 27) were recovered from apparently healthy cattle, whereas only three were from diarrhoeic animals (Eguale et al., 2016). In addition, *Salmonella* isolates obtained from faeces of healthy slaughtered beef cattle in Addis Ababa Abattoir (n = 20), from faeces of healthy birds in seven poultry farms (n = 26) in Addis Ababa and surrounding districts, and from faeces of pigs from four swine farms in Addis Ababa and Adaa district (n = 8) were used in this study. A geographic location from where *Salmonella* isolates were collected is shown in

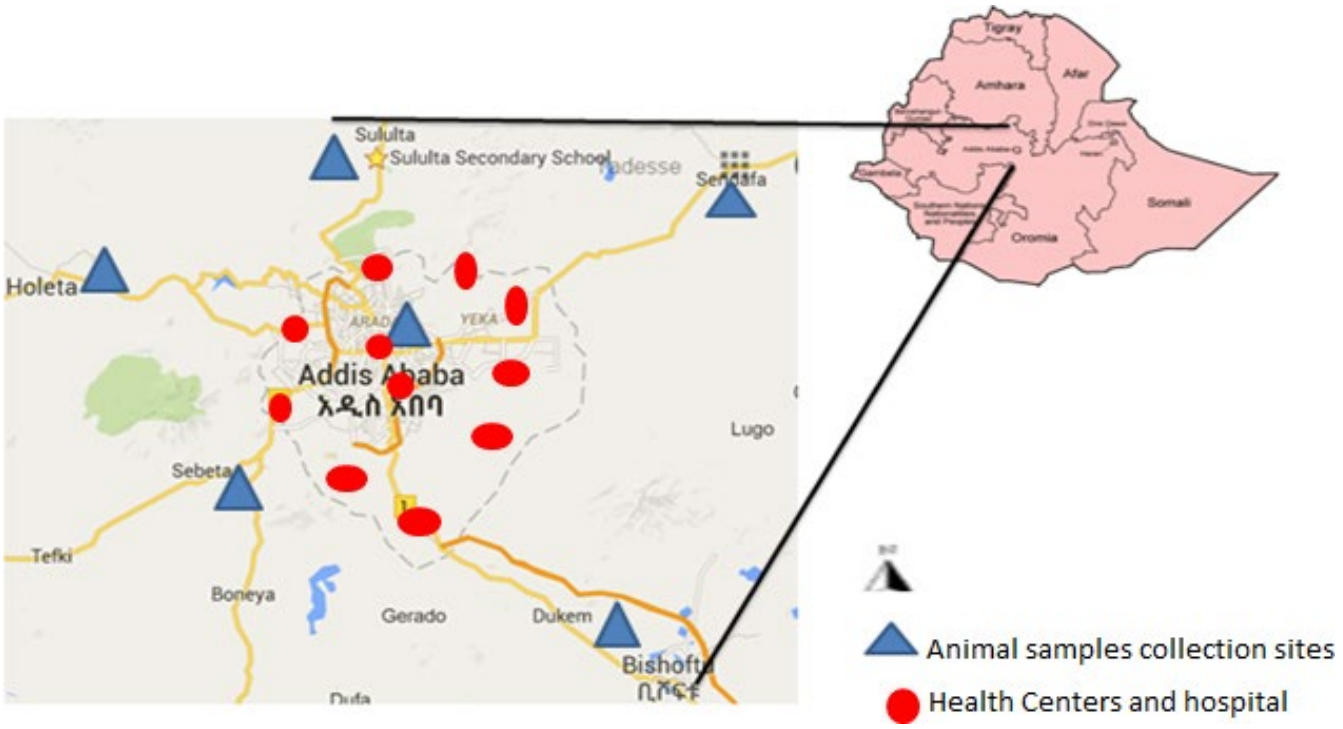

FIGURE 1 Locations in Addis Ababa and surrounding districts where *Salmonella* isolates from humans and animals were collected [Colour figure can be viewed at wileyonlinelibrary.com]

Figure 1. This study area serves as the source of food animal products for the human population in Addis Ababa and surrounding towns. Faecal samples from cattle and pigs were collected from rectum using an examination glove in to a sterile zippered plastic bag. In the same way, fresh faecal droppings of birds in poultry farms were collected in to zippered plastic bags. Human stool sample was collected into sterile plastic container. All samples were transported to the laboratory in an ice box within 3–4 hr of collection.

2.2 | Isolation, identification and serotyping of *Salmonella*

Salmonella isolation and identification were conducted according to WHO Global Foodborne Infections Network Laboratory Protocol (WHO, 2010). In brief, faeces were pre-enriched in buffered peptone water (BPW) (Becton Dickinson, Sparks, MD) in a ratio of 1:9 (weight by volume) and incubated overnight at 37°C. A 100 µl pre-enriched suspension was added into 9.9 ml of Rappaport-Vassiliadis enrichment broth (RVB; Oxoid, USA) and incubated at 42°C for 24 hr. At the same time, 1 ml of suspension was also transferred to 10 ml of Tetrathionate broth (TTB; Oxoid, USA) and incubated for 24 hr at 37°C. It was then streaked from both RV and TTB to Xylose Lysine Tergitol 4 (XLT-4; Oxoid, USA) selective agar, and the plates were incubated at 37°C for 24–48 hr. Presumptive *Salmonella* colonies were further investigated biochemically using triple sugar iron agar, urea, citrate and lysine iron agar slants. Isolates were further confirmed by genus-specific PCR (Cohen et al., 1993). Isolates were serotyped and phage typed at Public Health Agency of Canada, World Organization for Animal Health (OIÉ) Reference Laboratory for Salmonellosis, Guelph, Ontario, Canada, as described previously (Grimont & Weill, 2007). Phage typing was conducted only for *S*. Enteritidis, *S*. Heidelberg and *S*. Typhimurium (Anderson, Ward, Saxe, & Sa, 1977).

2.3 | Antimicrobial susceptibility testing

Susceptibility of the isolates to a panel of 18 antimicrobials was determined using the Kirby–Bauer disc diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2013). The following antimicrobials (Sensi-Discs, Becton, Dickinson and Company, Loveton, USA) and disc potencies (µg) were used: amikacin (30), amoxicillin + clavulanic acid (20/10), ampicillin (10), cefoxitin (30), ceftriaxone (30), cephalothin (30), chloramphenicol (30), ciprofloxacin (5), gentamicin (10), kanamycin (30), nalidixic acid (30), neomycin (30), nitrofurantoin (300), streptomycin (10), sulfisoxazole (1,000), sulfamethoxazole + trimethoprim (23.75/1.25), trimethoprim (5) and tetracycline (30). The interpretation of the categories of susceptible, intermediate or resistant was based on the CLSI guidelines (CLSI, 2013). Reference strain of *E. coli* ATCC 25922 was used as a quality control. Isolates resistant to more than one antimicrobials from different classes of antimicrobials were considered multidrug resistant (Brichta-Harhay et al., 2011).

2.4 | Genotyping using pulsed-field gel electrophoresis

Forty-seven isolates were systematically selected so as to represent major serovars isolated from food animals and humans, and genotyped

using PFGE to investigate genetic relatedness of *Salmonella* serovars from various food animals in Addis Ababa and surrounding districts and those obtained from diarrhoeic patients from Addis Ababa. Two out-group *Salmonella enterica* strains from Kenya and North Carolina, USA, both isolated from swine were also included in this analysis. PFGE was performed according to the Center for Disease Control and Prevention (CDC) PulseNet, as previously described (Ribot et al., 2006) using a contour-clamped homogeneous electric field (CHEF)-Mapper (Bio-Rad Laboratories, Hercules, CA). In brief, DNA digestion was performed using *Xba*I restriction enzyme. After staining with ethidium bromide, DNA fragments were visualized under UV trans-illumination (Gel Doc 2000, Bio-Rad Laboratories, Hercules, CA, USA). Gel images were photo documented using the Quantity One 1D analysis software (Bio-Rad Laboratories). PFGE gels were then analysed using BIONUMERICS software V. 4.61 (Applied Maths NV, Keistraat, Belgium) using dice coefficient similarity index and unweighted pair group method with arithmetic mean (UPGMA) cluster analysis. Image analysis was conducted based on 2.2% tolerance and 1.5% optimization. The plausible genetic threshold for clustering was 88%.

2.5 | Ethical consideration

The study protocol was ethically approved by the National Research Ethics Review Committee, Ministry of Science and Technology, Federal Democratic Republic of Ethiopia (Permit#3–10/474/05 dated 29–03–2013). Individual oral informed consent was obtained from all adult participants and the parents or guardians of all children who participated in the study.

2.6 | Data analysis

Difference in level of MDR occurrence in humans and animals was tested by student *t* test and one-way analysis of variance (ANOVA). The difference between the means was considered significant at $p < 0.05$.

3 | RESULTS

3.1 | Relative distribution of *Salmonella* serovars from food animals and diarrhoeic human patients

Overall, 152 *Salmonella* isolates from cattle, poultry, swine and human belonging to 20 serovars were characterized in this study. The frequency of *Salmonella* serovars detected according to host species is shown in Table 1. *Salmonella* Typhimurium and *S.* Saintpaul were detected in all types of food animals and humans. The dominant serovars isolated from animals were *S.* Saintpaul, *S.* Typhimurium and *S.* Kentucky representing 38.1%, 17.9% and 9.5% of all isolates, respectively, whereas in humans, *S.* Typhimurium, *S.* Virchow and *S.* Kottbus were frequently detected, representing 39.7%, 30.9% and 10.3% of the isolates, respectively. The distribution of *Salmonella* in poultry was restricted to a few serovars while diverse serovars were seen

TABLE 1 *Salmonella enterica* serovars isolated from cattle, poultry, swine and humans in central Ethiopia, April 2013-March 2014

Salmonella serovar	Dairy cattle	Slaughtered cattle	Poultry	Swine	Animal total	Human	Total (%)
Typhimurium	7	4	3	1	15	27	42 (27.6)
Saintpaul	6	4	20	2	32	1	33 (21.7)
Virchow	5	2	–	–	7	21	28 (18.4)
Kentucky	5	1	2	–	8	2	10 (6.6)
Kottbus	–	1	–	–	1	7	8 (5.3)
Miami	–	–	–	2	2	3	5 (3.3)
Haifa	–	3	1	–	4	–	4 (2.6)
Braenderup	–	2	–	–	2	1	3 (2)
Dublin	3	–	–	–	3	–	3 (2)
Newport	–	–	–	–	–	2	2 (1.3)
Mikawasima	1	2	–	–	3	–	3 (2)
Livingstone var.14+	1	–	–	1	2	–	2 (1.3)
Aberdeen	1	–	–	–	1	–	1 (0.7)
Concord	–	–	–	–	–	1	1 (0.7)
Agona	–	1	–	–	1	–	1 (0.7)
Entertidis	–	–	–	–	–	2	2 (1.3)
Heidelberg	–	–	–	1	1	–	1 (0.7)
I:6,7,14:-:I,w	1	–	–	–	1	–	1 (0.7)
V:ROUGH-O:-:-	–	–	–	–	–	1	1 (0.7)
I:ROUGH-O:i:1,2	–	–	–	1	1	–	1 (0.7)
Total	30	20	26	8	84	68	152

in cattle and humans. Among *S.* Typhimurium, 11 different known definitive types (DTs) and two atypical phage types were identified. The dominant phage type was DT 126 (*n* = 8; 19.1%), followed by DT 193 (*n* = 7; 16.3%). In an interesting manner, all *S.* Typhimurium DT 126 were isolated from human patients, whereas other DTs were fairly distributed among other host species. The single *S.* Heidelberg in this study was phage Type 2, whereas both *S.* Enteritidis were atypical. *Salmonella* Saintpaul, although was the first and the second most dominant serovar isolated from poultry and cattle, respectively, only one *S.* Saintpaul was detected from human. *Salmonella* Virchow was the second dominant serovar isolated from humans, and among animals, it was recovered only from dairy and slaughtered cattle. *Salmonella* Kentucky though was of low proportion, it was detected from dairy and slaughtered cattle, poultry as well as humans (Table 1).

3.2 | Antimicrobial susceptibility of *Salmonella* isolates from food animals and diarrhoeic human patients

On the whole, 140 (92.1%) of the 152 *Salmonella* isolates were intermediately or fully resistant to one or more antimicrobials tested. These involved 50 (100%) of isolates from cattle, 25 (96.2%) of isolates from poultry, six (75%) of isolates from swine and 59 (86.8%) of isolates from humans. Among all antimicrobials tested, intermediate or full resistance was more common to streptomycin (81.6%), sulfisoxazole (55.9%), nitrofurantoin (44.7%), kanamycin (44.1%) and tetracycline (34.9%). However, full resistance was high for streptomycin 43 (28.3%), followed by cephalothin 35 (23%), ampicillin 34 (22.4%) and sulfisoxazole 31 (20.4%) (Table 2). The rate of occurrence of resistance to some antimicrobials is variable among the isolates collected from different sources. Frequency of resistance to streptomycin, sulfisoxazole, tetracycline, ampicillin and cephalothin in *Salmonella* isolates from animals is relatively high compared to that seen in humans. For instance, resistance to streptomycin ranged from 25% to 80.8% in isolates from food animals, whereas in human isolates, only 13.2% were resistant. In the same way, resistance to tetracycline ranged from 20% to 35% in isolates obtained from food animals while only 5.9% of human isolates were fully resistant to tetracycline. Resistance to chloramphenicol was detected in 38.5% of isolates obtained from poultry, most of these isolates belonged to *S.* Saintpaul. On the other hand, all isolates obtained from other food animals were susceptible to chloramphenicol, and only one (1.5%) isolate from human patients was fully resistant to chloramphenicol in the current study. This strain was *S.* Concord isolated from a hospitalized diarrhoeic child. Overall, rate of occurrence of resistance to antimicrobials in *Salmonella* isolates from human was less common compared to isolates obtained from food animals (Table 2).

Mean ± *SEM* number of antimicrobials to which *Salmonella* isolates obtained from slaughtered cattle, dairy cattle, poultry, swine and human patients demonstrated intermediately or fully resistant was 5.1 ± 0.42, 5.33 ± 0.7, 5.69 ± 0.64, 2.88 ± 0.74 and 3.1 ± 0.35, respectively. *Salmonella* isolates obtained from slaughtered cattle, dairy cattle and poultry were resistant to greater numbers of antimicrobials compared to those obtained from humans ($p < 0.05$) while no significant difference was observed among isolates obtained from food animals (Figure 2).

3.3 | Resistance pattern of *Salmonella* isolates from food animals and humans

Diverse phenotypic resistance patterns were detected among *Salmonella* serovars isolated from food animals and humans. The single *S.* Concord isolated from the hospitalized child was MDR to several drugs (AmpAmcCCroCfFoxSxtTmpS). Unlike *S.* Concord, most of less prevalent isolates in the current study did not exhibit resistance to several drugs. For instance, among the four *S.* Haifa strains, only one isolate from poultry exhibited resistance to eight antimicrobials (KSmtTmpTeSuSNitroNA), whereas most of the other strains belonging to serotypes with low prevalence were resistant to less number of antimicrobials. Full susceptibility to all antimicrobial agents tested was more commonly detected in human isolates (*n* = 9; 13.2%) than isolates from animals (*n* = 3; 3.6%; data not shown). All *S.* Kentucky isolated from animals and humans were MDR to several antimicrobials. All of them have shared resistance phenotype (AmpCfCipTeSuSNa). Among the dominant serovars, variable resistance patterns were detected for *S.* Typhimurium, while majority of *S.* Saintpaul isolated from food animals were resistant to several antimicrobials (Table 3).

3.4 | Genetic diversity of representative *Salmonella* isolates using PFGE

Pulsed-field gel electrophoresis analysis in this study showed large genotypic diversity with 11 genotypic clusters and seven sporadic clones (Figure 3). The majority of the *Salmonella* isolates within a serovar were clustered together. All *S.* Virchow isolates from dairy cattle (*n* = 2), slaughtered cattle (*n* = 1) and diarrhoeic human patients (*n* = 5) in Addis Ababa formed a single cluster. This cluster is further subclustered into two. The first group consisted of isolates from diarrhoeic patients (*n* = 4) and dairy cattle (*n* = 2) in Addis Ababa with an indistinguishable PFGE profile while the second consisted of one isolate from a diarrhoeic human patient and the other from slaughtered cattle.

Two *S.* Kottbus strains obtained from two diarrhoeic patients from two separate health centres in Addis Ababa clustered together while another *S.* Kottbus strain from slaughtered cattle in Addis Ababa was distantly related to these strains. *Salmonella* Braenderup isolated from slaughtered cattle and diarrhoeic patient in Addis Ababa also clustered together. *Salmonella* Kentucky isolated from slaughtered cattle in Addis Ababa abattoir (*n* = 1), poultry from Adaa district (*n* = 1), dairy cattle from Addis Ababa (*n* = 1) and diarrhoeic human patient in Addis Ababa (*n* = 1) clustered together. In addition, all of these isolates shared common MDR phenotype.

Six of the eight *S.* Saintpaul examined by PFGE in the current study isolated from poultry, dairy cattle and swine clustered together while one *S.* Saintpaul from a diarrhoeic patient and one

TABLE 2 Antimicrobial resistance profile of *Salmonella* isolates from different food animals and diarrhoeic human patients, April 2013–March 2014

	Dairy cattle (*n* = 30)		Slaughtered cattle (*n* = 20)		Poultry (*n* = 26)		Swine (*n* = 8)		Human (*n* = 68)		Total (*n* = 152)		
Antimicrobial agent	**No. (%I)**	**No. (% R)**	**No. (%I)**	**No. (%R)**	**No. (% I)**	**No. (%R)**	**No. (%I)**	**No. (%R)**	**No. (%I)**	**No. (%R)**	**No. (%I)**	**No. (%R)**	**I + R (%)**
An	–	–	6 (30)	–		–	–	–	–	–	6 (3.9)	–	6 (3.9)
Amp	1 (3.3)	9 (30)	1 (5)	5 (25)	–	11 (42.3)	–	1 (12.5)	2 (2.9)	8 (11.8)	4 (2.6)	34 (22.4)	38 (25)
Amc	5 (16.7)	4 (13.3)	–	3 (15)	6 (23.1)	6 (23.1)	1 (12.5)	–	2 (2.9)	5 (7.4)	14 (9.2)	18 (11.8)	32 (21.1)
Cf	5 (16.7)	9 (30)	2 (10)	5 (25)	1 (3.8)	11 (42.3)	–	1 (12.5)	2 (2.9)	9 (13.2)	9 (5.9)	35 (23)	44 (28.9)
C	–	–	–	–	1 (3.8)	10 (38.5)	–	–	1 (1.5)	1 (1.5)	2 (1.3)	11 (7.2)	13 (8.6)
Cro	–	–	–	–	1 (3.8)	–	–	–	1 (1.5)	1 (1.5)	2 (1.3)	1 (0.7)	3 (2)
Fox	–	–	–	–	–	–	1 (12.5)	–	1 (1.5)	1 (1.5)	2 (1.3)	1 (0.7)	3 (2)
Cip	4 (13.3)	5 (16.7)	2 (10)	1 (5)	3 (11.5)	2 (7.7)	2 (25)	–	1 (1.5)	2 (2.9)	12 (7.9)	10 (6.7)	22 (14.5)
Gm	1 (3.3)	6 (20)	1 (5)	1 (5)	–	2 (7.7)	–	–	2 (2.9)	3 (4.4)	4 (5.9)	12 (7.9)	16 (10.5)
K	14 (46.7)	–	15 (75)	–	12 (46.2)	–	3 (37.5)	–	22 (32.4)	1 (1.5)	66 (43.4)	1 (0.7)	67 (44.1)
Tmp	–	1 (3.3)	–	2 (10)	–	1 (3.8)	–	–	–	3 (4.4)	–	7 (4.6)	7 (4.6)
Sxt	–	–	–	2 (10)	–	1 (3.8)	–	–	–	3 (4.4)	–	6 (3.9)	6 (3.9)
Te	10 (33)	6 (20)	7 (35)	7 (35)	1 (3.8)	8 (30.8)	5 (62.5)	–	5 (7.4)	4 (5.9)	28 (18.4)	25 (16.5)	53 (34.9)
Su	10 (33)	8 (26.7)	12 (60)	5 (25)	11 (42.3)	13 (50)	–	–	21 (30.9)	5 (7.4)	54 (35.5)	31 (20.4)	85 (55.9)
S	18 (60)	8 (26.7)	13 (65)	5 (25)	3 (11.5)	21 (80.8)	6 (75)	–	41 (60.3)	9 (13.2)	81 (53.3)	43 (28.3)	124 (81.6)
Nitro	8 (26.7)	10 (33)	9 (45)	6 (30)	3 (11.5)	2 (7.7)	2 (25)	1 (12.5)	24 (35.3)	3 (4.4)	46 (29.6)	22 (14.5)	68 (44.7)
Na	3 (10)	6 (20)	–	1 (5)	2 (7.7)	3 (11.5)	–	2 (25)	5 (7.4)	2 (2.9)	10 (6.6)	14 (9.2)	24 (15.8)
N	2 (6.7)	3 (10)	4 (20)	–	3 (11.5)	–	–	–	8 (11.8)	1 (1.5)	17 (11.2)	4 (2.6)	21 (13.8)

Notes. An: amikacin; Amp: ampicillin; Amc: amoxicillin and clavulanic acid; Cf: cephalothin; C: chloramphenicol; Cro: ceftriaxone; Cip: ciprofloxacin; Fox: cefoxitin; Gm: gentamicin; K: kanamycin; Tmp: trimethoprim; Sxt: sulfamethoxazole + trimethoprim; Te: tetracycline: Su: sulfisoxazole; S: streptomycin; Nitro: nitrofurantoin; Na: nalidixic acid; N: neomycin; I: intermediately resistant; R: resistant.

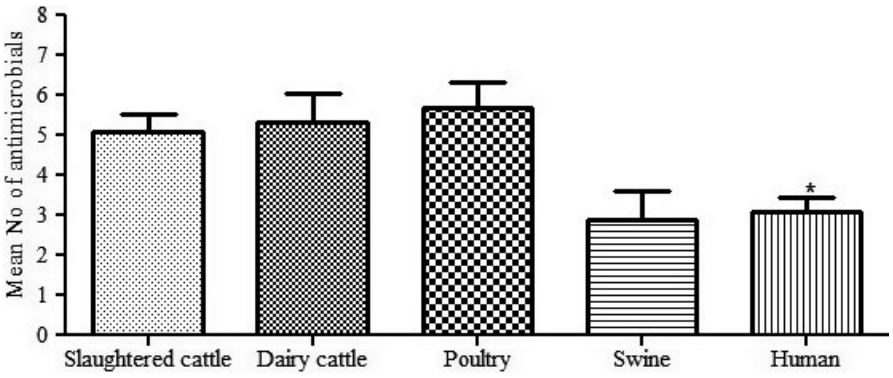

FIGURE 2 Level of multidrug resistance among *Salmonella* isolates from different food animals in central Ethiopia and diarrhoeic human patients in Addis Ababa (Each bar represents mean ± *SEM* number of antimicrobials to which isolates were resistant)

TABLE 3 Antimicrobial resistance pattern of dominant *Salmonella* serovars isolated from food animals and humans in central Ethiopia, April 2013-March 2014

	Source					
***Salmonella* serovar**	**Cattle**	**Poultry**	**Swine**	**Human**	**Total**	**Antimicrobial resistance pattern and host**
Kentucky	6	2	–	2	10	AmpAmcCfCipTeSuSNa(1C), AmpCfCipGmTmpTeSuSNitroNaN(1C), AmpCfCipGmTeSuSNaN(C), AmpAmcCfCipGmTeSuSNa(C), AmpCfCipGmTeSuSNa(C), AmpAmcCfCipGmTeSuSNa (C), AmpAmcCfCipFoxGmTeSuSNa(H), AmpCfCipGmTeSuSNa(H), AmpAmcCfCipGmTeSuSNa(p), AmpAmcCfCipGmTeSuSNa(P)
Saintpaul	10	20	2	1	33	CCroNa(1P), AmpCTeSu(1P), AmpCSu(1P), AmpCTe Su(1P) AmpCf Te(1C), AmpCCfNitro(1P), AmpC CfTeSu(1P) AmpCfTe(1C), SuNitro(1C), Nitro(1C), AmpAmcCSu(1P) AmpAmcCSu(2P), SuNitro(1C), Su(1P,) SuNitro(1P) Nitro(1C), AmpAmcCCfTeSu(1P)
Typhimurium DT At	1	–	–	3	4	–
Typhimurium DT1	–	1	–	5	6	–
Typhimurium DT 2		1	–	3	4	–
Typhimurium DT 3	1	–	–	4	5	AmpAmcCf(1C), AmpAmcCf(2H), AmpCf(1H),
Typhimurium DT 4	1	–	–	–	1	AmpAmcCf (C)
Typhimurium DT 66	–	1	–	1	2	TeSuS(1H, 1P)
Typhimurium DT 67	1	–	–	–	1	–
Typhimurium DT 74	–	–	1	–	1	–
Typhimurium DT 104	1	–	–	–	1	–
Typhimurium DT 126	–	–	–	8	8	Nitro(1H)
Typhimurium DT 193	1	–	–	3	4	AmpCfKSxtTmpTesuSN(1H), SxtTmpTeS(1C)
Typhimurium Var. Copenhagen DT 193	3	–	–	–	3	–
Typhimurium Var. Copenhagen DT At	1				1	–
Typhimurium Var. Copenhagen DT U285	1				1	–
Virchow	7	–	–	21	28	Amp(1C), AmpCfCipGmTeSuSNa(1C), AmpAmcCfS(1C) AmpAmcCf(1H),(Nitro(1C)

Notes. An: amikacin; Amp: ampicillin; Amc: amoxicillin and clavulanic acid; C: chloramphenicol; Cf: cephalothin; Cip: ciprofloxacin; Cro: ceftriaxone; Fox: cefoxitin Gm: gentamicin; K: kanamycin; Na: nalidixic acid; Tmp: trimethoprim; Te: tetracycline; Su: sulfisoxazole; S: streptomycin; Nitro: nitrofurantoin; Sxt: sulfamethoxazole + trimethoprim; N: neomycin; DT: definitive type; At: atypical; H: human; C: cattle; S: swine; P: poultry; –: isolate is either fully susceptible or intermediately resistant to all antimicrobials tested.

from slaughtered cattle in Addis Ababa showed a different PFGE fingerprint with a very distant genetic relationship. Those *S.* Saintpaul strains clustered together were from food animals in Adaa district except two isolates obtained from swine from Addis Ababa. *Salmonella* Miami isolated from swine and human patient in Addis Ababa also showed a related PFGE profile.

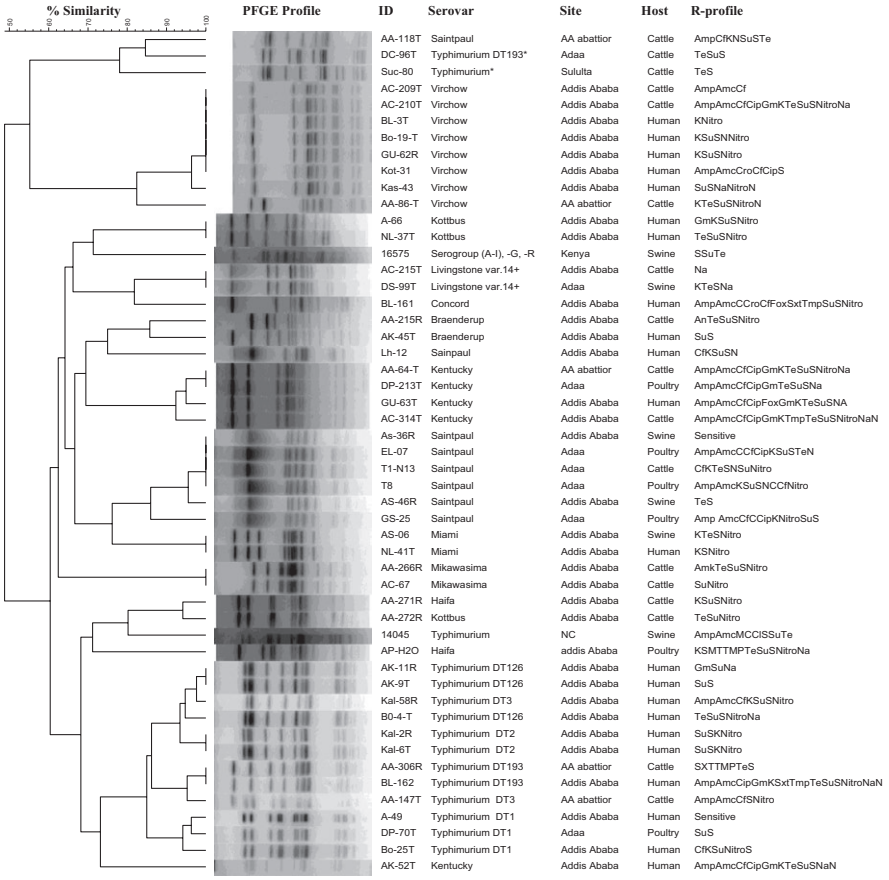

FIGURE 3 PFGE dendrogram showing genotypic similarity among *Salmonella* serovars isolated from humans, cattle, poultry and pigs in central Ethiopia. Amc: amoxicillin + clavulanic acid; Amp: ampicillin; AR: antimicrobial resistance; C: chloramphenicol; Cf: cephalothin; Cip: ciprofloxacin; Cro: ceftriaxone; DT: definitive phage type: Amk: amikacin; Gm: gentamicin; K: kanamycin; N: neomycin; Na: nalidixic acid; NC: North Carolina; Nitro: nitrofurantoin; PFGE: pulsed-field gel electrophoresis; R-profile: resistance profile; S: streptomycin; Su: sulfisoxazole; Te: tetracycline; Tmp: trimethoprim

Strains of *S.* Typhimurium, the predominant serovar shared by food animals and humans, were grouped into three genotypic clusters and three sporadic clones. The first cluster involved only isolates from humans, while the 2nd and 3rd cluster involved isolates from both humans and animals. Among isolates in the second cluster, strain BL-162 (DT193) isolated from a diarrhoeic child from Tikur Anbessa Specialized Hospital (TASH) showed completely identical PFGE profile with strain AA-306 (DT193) isolated from faeces of slaughtered cattle at Addis Ababa slaughterhouse. In same way, in the third cluster, the fingerprint pattern of two DT1 isolates from diarrhoeic patients and one from poultry looks like an indistinguishable.

4 | DISCUSSION

In the current study, *Salmonella* serovars frequently isolated from clinical human patients such as *S.* Typhimurium, *S.* Virchow, *S.* Kottbus and *S.* Kentucky were also isolated from spatially and temporally related food animals. *Salmonella* Saintpaul, although it was the most frequently isolated serovar from food animals during the study period, only one *S.* Saintpaul was recovered from a diarrhoeic patient in Addis Ababa. The possible reason is as most of the strains of *S.* Saintpaul in the current study were isolated from poultry and dairy farms located in Adaa district, these strains might have been circulating only in this specific region and could not get access to the patients in Addis Ababa involved in the current study, or the strains circulating in animals in this region might be less virulent to human. In an interesting manner, genotyping by PFGE revealed that while majority of strains of *S.* Saintpaul isolated from food animals were clonally related, the single *S.* Saintpaul isolated from diarrhoeic human patient was distantly related to isolates obtained from food animals, suggesting another sources of infection. The fact that most of the isolates from poultry were *S.* Saintpaul and were all obtained from farms in Adaa district suggest possibility of dissemination of this strain across the farms in the town. Most of the farms in this town receive the day-old chickens as well as poultry feed from a common source (personal communication).

Salmonella Saintpaul was previously reported from camel (Molla, Salah, Alemayehu, & Mohammed, 2004) and minced beef (Zewdu & Cornelius, 2009) in the country. Another recent study also showed that *S.* Saintpaul was the dominant serovar in beef abattoir and beef processing plant in Addis Ababa(Hiko, Irsigler, Ameni, Zessin, & Fries, 2016).

Diverse antimicrobial susceptibility phenotypes were observed among *Salmonella* serovars isolated from different sources. Three *S.* Typhimurium DT3 isolates from diarrhoeic human patients and one *S.* Typhimurium DT3 from cattle had a common resistance profile (AmpAmcCf). In addition, some of the *S.* Typhimurium isolates from human and animal origin showed a closely related PFGE

fingerprint suggesting the possibility of source of infection of human cases from cattle. One of the four *S*. Typhimurium DT193, isolated from a hospitalized diarrhoeic child in TASH, was resistant to several antimicrobials unlike the other two human isolates from diarrhoeic patients at primary health centres which were pansusceptible to all antimicrobials tested and one isolate from cattle was resistant to four antimicrobials. Strain of MDR *S*. Typhimurium DT193 obtained from the hospital might be due to nosocomial infection which acquired resistance due to frequent exposure to different antimicrobials within the hospital. A previous study in TASH showed high loads of MDR nosocomial pathogens including *Salmonella* carried by cockroaches in a neonatal intensive care unit (Tilahun et al., 2012), and another study also showed high mortality from blood stream infection in TASH due to MDR enterobacteriaceae (Seboxa et al., 2015).

The second dominant serovar isolated from human patients in the current study, *S*. Virchow was also commonly detected in dairy cattle and slaughtered cattle collected during similar study period in Addis Ababa. In an interesting manner, all *S*. Virchow isolates in the current study were isolated from cattle and clinical diarrhoeic patients residing in Addis Ababa. Most of these strains have common antimicrobial resistance profiles and representative isolates from different hosts also showed closely related PFGE fingerprint, suggesting probability of clonal spread of the strain in Addis Ababa. In the same way, *S*. Virchow was reported to be the second dominant NTS serovar in human patients in Israel (Weinberger et al., 2006). It is also among the top *Salmonella* serovars causing human salmonellosis in Europe (Bonalli et al., 2011). There is a need for appropriate control strategy to reduce spread of this pathogen in Addis Ababa and surrounding districts.

Salmonella Kottbus was also one of the dominant serovars detected from human patients in Addis Ababa. Among food animals, only a single *S*. Kottbus was detected from faeces of slaughtered cattle. Like *S*. Saintpaul mentioned above, this strain was not genotypically related to the two *S*. Kottbus strains isolated from diarrhoeic patients in Addis Ababa. *Salmonella* Kottbus was previously reported from apparently healthy camels (Molla et al., 2004) and pork in Ethiopia (Zewdu & Cornelius, 2009). Although we do not have data on previous occurrence of human *S*. Kottbus infection in the country, this serovar has been reported to cause serious multistate outbreaks of human salmonellosis in other countries (Palmera-Suárez, García, García, Barrasa, & Herrera, 2007; Winthrop et al., 2003).

Occurrence of *S*. Kentucky in poultry, dairy cattle, slaughtered cattle as well as clinical human patients in Addis Ababa together with observed shared MDR phenotype and genotypic relatedness of selected strains shown by PFGE analysis suggests clonal spread across various host species in the study area. In an interesting manner, all *S*. Kentucky strains in the current study were isolated from the Addis Ababa city limit and were resistant to seven antimicrobials in common (AmpCfCipTeSuSNa). Resistance to quinolones in all of these isolates was shown to be due to double mutation in *gyr*A and *par*C genes in our previous study (Eguale et al., 2017). MDR *S*. Kentucky strains belonging to a single clone (ST198) resistant to quinolones were previously reported from European travellers returning from different African and Asian countries (Le Hello et al., 2011). MDR *S*. Kentucky was also previously isolated from beef, chicken and pork in Ethiopia (Molla et al., 2007). Circulation of such MDR and persistent strains in a highly populated city like Addis Ababa is a major threat to public health and requires serious attention.

The overall frequency of resistance to most of the antimicrobials and especially to tetracycline and sulfisoxazole was higher in *Salmonella* isolates from food animals compared to those obtained from clinical human patients. The possible reason for this could be due to frequent use of these antimicrobials in farms favouring selection of resistant strains. Our previous study also showed that antimicrobials such as oxytetracycline, streptomycin and sulphonamides are widely used in dairy farms in the Addis Ababa and surrounding districts (Eguale et al., 2016). The other reason could be the fact that humans can also be infected with *Salmonella* from other sources including meat from small ruminants and small-scale backyard chicken with little exposure to veterinary services including antimicrobial agents (Sambo et al., 2015). The significantly higher occurrence of MDR in *Salmonella* isolates from food animals compared to those from humans entails high risk of transmission of antimicrobial-resistant isolates and resistance genetic markers to humans from these food animals.

Salmonella Miami from swine and diarrhoeic human patients and *S*. Braenderup from cattle and diarrhoeic human patients also showed indistinguishable PFGE fingerprints. This recovery of similar serovars in humans and animals as well as the occurrence of related multidrug resistance profiles especially in *S*. Virchow and *S*. Kentucky suggests the possibility of transfer of *Salmonella* and their antimicrobial resistance genetic markers from these food animals to humans or vice versa.

In general, despite wide diversity, there is clear indication that similar or closely related genotypes of *Salmonella* are circulating among humans and animals. In particular, *S*. Virchow, *S*. Typhimurium and *S*. Kentucky were found to circulate among food animals and humans in the study area. Of particular concern is detection of clonally related MDR *S*. Kentucky in dairy, slaughtered cattle, poultry and humans; MDR *S*. Virchow in dairy cattle, slaughtered cattle and humans. However, as the current isolates were obtained from unrelated clinical patients from various primary health centres without any reported outbreak and with little information on patients exposure to specific food animal products and the absence of clear epidemiological linkage, there is a probability that isolates with identical PFGE profile might not be an indicator of clonality of the isolates. This limitation should be taken in to account when interpreting the findings of this study. The fact that animals and humans live in close proximity in the study area in the absence of strong biosecurity poses a major public health problem. Therefore, integrated surveillance of *Salmonella* serovars in humans and animals and implementation of appropriate pathogen control strategies along critical points in food animal production from farm to bench is recommended.

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CONFLICT OF INTEREST

None.

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