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ORIGINAL RESEARCH

# Prevalence and antimicrobial resistance profiles of *Salmonella enterica* serovars isolated from slaughtered cattle in Bahir Dar, Ethiopia

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**Abstract** A study was undertaken from October 2006 to March 2007 to determine the prevalence and antimicrobial resistance patterns of *Salmonella* serovars. Liver, mesenteric lymph nodes, intestinal content, and carcass swab samples (each $n=186$) were collected from 186 apparently healthy slaughtered cattle at Bahir Dar abattoir. Bacteriological analysis was done according to the International Organization for Standardization (ISO 6579 2002). Isolates were serotyped at Agence Française de Securite Sanitaire des Aliments, Cedex, France. Twenty-eight isolates consisting of *Salmonella* Typhimurium, *Salmonella* Newport, *Salmonella* Haifa, *Salmonella* Heidelberg, *Salmonella* Infantis, and *Salmonella* Mishmarhaemek were identified. *Salmonella* Typhimurium and *Salmonella* Newport were most frequently isolated while *Salmonella* Heidelberg and *Salmonella* Mishmarhaemek were isolated least. Eleven of the 28 (39.3%) were resistant to one or more of the antimicrobials tested. Resistance was shown to ampicillin, chloramphenicol, gentamycin, norfloxacin, polymyxin-B, streptomycin, tetracycline, and trimethoprim. Four of 11 (36.4%) were multiple antimicrobial resistant. All the isolates tested were susceptible to the antimicrobial effects of gentamycin, norfloxacin, and trimethoprim. Eleven, four, and two isolates of the 28 were resistant to streptomycin, tetracycline, and ampicillin, respectively. All isolates of *Salmonella* Infantis, *Salmonella* Typhimurium (except one), and *Salmonella* Mishmarhaemek were susceptible to the tested antimicrobials. One Typhimurium isolate was resistant to chloramphenicol, streptomycin, and tetracycline. *Salmonella* Haifa was multiply antimicrobial resistant to ampicillin, tetracycline, and streptomycin. All isolates of *Salmonella* Heidelberg were resistant to streptomycin. Results of this study indicated high level of carcass contamination with antimicrobial-resistant *Salmonella* serovars which could pose public health risk; suggests need for hygienic slaughtering operations and proper cooking of meat before consumption. Further detailed studies involving different abattoirs, animal products, food items, and animals on different settings were recommended in the study area.

**Keywords** Antimicrobial resistance · Cattle · *Salmonella* · Serovars · Ethiopia

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## Introduction

Salmonellosis is the leading most common foodborne zoonoses (Acha and Szyfres 2001; Maddox 2003) caused by organisms of the genus *Salmonella* (Radostits et al. 2007). Non-typhoidal *Salmonella* represents an important human and animal pathogen worldwide (Hoelzer et al. 2011). Infection in animals is of importance because of the direct economic effect. Of even greater importance is that animals constitute a vast reservoir of these organisms for human infection (Libby et al. 2004). In humans, in addition to concern about foodborne zoonoses caused by *Salmonella* organisms, concern has also been raised about the impact of acquired antimicrobial resistance transferred among these organisms (Dargatz et al. 2003) which limits therapeutic

options both in veterinary and public health practice. Most human salmonellosis cases are foodborne (Hoelzer et al. 2011). Dahshan et al. (2011) indicated chance of transmission of antimicrobial-resistant *Salmonella* to humans through the food chain and a threat to human health in their study of emergence of multidrug-resistant *Salmonella* Stanley from cattle diagnostic specimens in southern Japan. In Ethiopia, Beyene et al. (2011) detected multiply drug-resistant *Salmonella* organisms in their study on etiology of febrile and diarrheic illness in Ethiopian children focusing on *Salmonella*. Multidrug-resistant *Salmonella* Concord infections was isolated in Europe and the USA in children adopted from Ethiopia (Hendriksen et al. 2009).

However, it is often very difficult to predict when and why a *Salmonella* serovar will decline or rise in importance, either as a result of natural causes or control measures, which indicates the need for continuous attention concerning the distribution and importance of *Salmonella* serovars involved. In Ethiopia, *Salmonella* serovars have been reported from slaughtered cattle and cattle products (Nyeleti et al. 2000; Alemayehu et al. 2003; Sibhat et al. 2009). Beyene et al. (2011) reported different *Salmonella* isolates from febrile and diarrhoeic children. According to Mache (2002), *Salmonella* was one of the major causes of diarrhea in humans.

This together with tradition of raw meat consumption and indiscriminate use of antimicrobials signifies the importance of salmonellosis in the country. Therefore, this study was designed to find out diversity and antimicrobial resistance patterns of *Salmonella* serovars isolated from apparently healthy slaughtered cattle at Bahir Dar municipality abattoir, north-west Ethiopia.

## Materials and methods

### Study animals and sampling

This cross-sectional study was conducted on 186 apparently healthy adult male cattle that have finished their traction life and slaughtered between October 2006 and March 2007 at Bahir Dar municipality abattoir. Study animals were indigenous zebu cattle kept in extensive management system originating from west Gojam and South Gondar Zones of Amhara National Regional State; and some of them were kept for 24 h to 15 days at Bahir Dar before being presented to the abattoir for slaughter. They were kept in open-air during the day and in barns during the night and fed hay and water by their respective owners until they were presented for slaughter. There was also an opportunity for some animals to be bought and presented to the abattoir for slaughter on the same day. Animals were kept without feed and water in holding pens for an average of 7 h after they were presented to the abattoir. Animals were eviscerated in the same room where they were stunned, there was a separate building for managing and storing of hides. Except the inspection activity, a separate group each containing two slaughter men was assigned to carry out all the slaughtering process of one animal from stunning to evisceration, managing all the visceral organs and finally to loading. In the slaughter house, there was tap water which was not warmed. The wall of the slaughter house was rough while the floor was smooth which had drainage at the center. A knife was used to handle the slaughtering process of one or more animals. There was no significant washing of the hands and among the slaughtering processes. Loading was based on manual means.

On each sampling day, study animals were selected randomly by using the identification numbers given to the animals both for antemortem and postmortem examination; then liver tissue, mesenteric lymph nodes, intestinal content and carcass swab samples were aseptically collected in sterile containers. Carcass swab samples were collected by rubbing the carcass using sterile cotton swabs moistened in 10-ml buffered peptone water (BPW; AES, Cedex, France) at the end of slaughtering process. Samples were stored at 4°C for not more than 12 h until they are processed in Bahir Dar Regional Veterinary Laboratory.

### Isolation and identification of *Salmonella*

Isolation and identification was made based on the recommendations of the ISO method for the detection of *Salmonella* from food and animal feeding stuffs (ISO 6579 2002). Twenty-five grams from each of lymph node and liver were minced into fine pieces and placed in a separate stomacher bag containing 225-ml BPW and homogenized using stomacher. Carcass swab and intestinal content samples were homogenized by shaking manually. After all homogenized samples were incubated at 37°C for about 16 h, a portion of the culture (0.1 ml) was transferred to a tube of selective enrichment liquid media containing 10 ml of a Rappaport–Vassiliadis with soya broth (Titan Biotech, Raj, India) and 1 ml to a tube containing 10 ml of Muller–Kauffmann tetrathionate novobiocin broth (Oxoid, Hampshire, England) and incubated at 42°C for 24±3 h and at 37°C for 24±3 h, respectively. A loopful of inoculum from each of enrichment cultures were inoculated on the surface of two different plates containing xylose lysine deoxycholate (XLD) agar (AES laboratoire, Cedex, France) and MacConkey agar and were incubated at 37°C for 24±3 h.

For confirmation, five presumptive *Salmonella* colonies both from XLD agar and MacConkey agar were selected and streaked onto the surface of pre-dried nutrient agar (Oxoid, Hampshire, England) plates and incubated at 37°C for 24±3 h. If there were less than five typical colonies on

one plate, all the typical or suspect colonies were used. Pure cultures from the nutrient agar were used for biochemical confirmation. Triple sugar iron agar (Difco, Becton Dickinson, Claix, France), lysine iron agar (Difco™, Becton Dickinson, Claix, France), urea agar (BBL®, Becton Dickinson, USA) and Simons's citrate agar (Difco, Detroit, USA) were inoculated and incubated at 37°C for 24±3 h. Biochemical confirmation of *Salmonella* organisms were made according to Quinn et al. (1999).

*Serotyping* Biochemically confirmed isolates were cultured on brain heart infusion agar (Difco, Becton Dickinson, Claix, France) and shipped to Agence Française de Securite Sanitaire des Aliments, Maisons-Alfort, Cedex, France for serotyping.

### Antimicrobial susceptibility testing

Each isolate was tested for susceptibility to eight commonly used antimicrobials using the disk diffusion method according to guidelines set by the National Committee for Clinical Laboratory Standards (NCCLS 1997). Five milliliter tryptic soy broth (Oxoid, England) was inoculated and incubated at 35°C for 4 h. Culture of each isolate was compared with 0.5 McFarland turbidity standards (if necessary adjusted by adding sterile saline into tubes). Muller Hinton agar plates (Difco, Becton Dickinson, Claix, France) were inoculated by swabs immersed in each of the culture and held at room temperature for 30 min to allow drying. Antimicrobial impregnated discs were dispensed on the surface of cultures of Muller Hinton agar and incubated at 37°C for 20 h. The diameters of the zones of inhibition were recorded to the nearest millimeter and classified as resistant, intermediate, or susceptible according to published interpretive chart (NCCLS 1997). Tested antimicrobials, their concentration in the discs and their zone of inhibition in deciding susceptibility are given in Table 1.

## Results

The prevalence of *Salmonella* at animal and sample level was observed. At animal level, overall prevalence of 7% (13 of 186) was bacteriologically positive for *Salmonella* (Table 2). At sample level, *Salmonella* was observed with prevalence of 3.8% (28 of 744). *Salmonella* was detected from liver, mesenteric lymph nodes, carcass swab, and intestinal content samples (each $n=186$) with prevalence of 1.1%, 3.2%, 4.8%, and 5.9%, respectively. Six different serovars of *Salmonella* and four untypable (rough strains) were isolated (Table 2). *Salmonella* Typhimurium and *Salmonella* Newport were isolated at the highest frequency; *Salmonella* Heidelberg and *Salmonella* Mishmarhaemek were isolated at frequency of 7.1% (2 of 28) of the total isolates.

More than one isolate was detected in 11 of the animals which were positive for *Salmonella.* All the six *Salmonella* serovars were detected both from mesenteric lymph node, intestinal content, and carcass swab samples except *Salmonella* Mishmarhaemek and *Salmonella* Heidelberg, which were not detected from samples of mesenteric lymph nodes (Table 2).

### Antimicrobial resistance profiles

Of the 28 isolates, 39.3% were resistant to streptomycin. All isolates were susceptible to the antimicrobial effects of gentamycin, norfloxacin, and trimethoprim. Antimicrobial resistance was detected in 11 of the 28 isolates (39.3%), of which four of 28 (14.3%) were multiple antimicrobial resistant (MAR) and was higher in isolates from the mesenteric lymph nodes while seven of 28 (25%) were resistant to a single antimicrobial agent. The highest number of resistant isolates was detected from intestinal contents (Table 3).

## Discussion

The highest sample prevalence (5.9%) was found on intestinal content followed by carcss (4.8%). The prevalence of *Salmonella* from carcasses was in agreement with the work of Alemayehu et al. (2003) who reported prevalence of 3.1% and 2.8% from muscles of the diaphragm and the abdomen, respectively. Fegan et al. (2004) also reported carcass contamination of 2% from an abattoir in Australia. In any case, carcass contamination levels have to be taken with caution; as the presence of even a single carrier animal can be a potential source of contamination of the carcasses, environment or personnel.

*Salmonella* serovars including *Salmonella* Typhimurium, *Salmonella* Newport, *Salmonella* Infantis, *Salmonella* Haifa, *Salmonella* Heidelberg, and *Salmonella* Mishmarhaemek were detected. *Salmonella* Typhimurium and *Salmonella* Newport were the most frequently isolated serovars, each accounting for 21.4% of the total isolates. The frequency of isolation of *Salmonella* Typhimurium was consistent with the report of Alemayehu et al. (2003) and Fegan et al. (2004) who found *Salmonella* Typhimurium as being the dominant serovar among their isolates. *Salmonella* Infantis and *Salmonella* Mishmarhaemek were detected with prevalences of 17.9% and 7.1%, respectively. *Salmonella* Heidelberg was detected with prevalence equal to that of *Salmonella* Mishmarhaemek.

**Table 1** Antimicrobials and their concentrations used to test susceptibility of isolates

| Antimicrobial agent | Symbol | Amount/disk | Antimicrobial agent | | |
|---|---|---|---|---|---|
| | | | Resistant intermediate susceptible | | |
| Ampicillin | Amp | 10 μg | ≤13 | 14–16 | ≥17 |
| Chloramphenicol | Chl | 30 μg | ≤12 | 13–17 | ≥18 |
| Gentamycin | Gen | 10 μg | ≤12 | 13–14 | ≥15 |
| Norfloxacin | Nor | 10 μg | ≤12 | 13–16 | ≥17 |
| Polymyxin-B | Pol | 300 IU | ≤8 | 9–11 | ≥12 |
| Streptomycin | Str | 10 μg | ≤11 | 12–14 | ≥15 |
| Tetracycline | Tet | 30 μg | ≤14 | 15–18 | ≥19 |
| Trimethoprim | Tri | 5 μg | ≤10 | 11–15 | ≥16 |

*Salmonella* Typhimurium was the second dominantly isolated serovar in a study on etiology of febrile and diarrheic illness in Ethiopian children focusing on *Salmonella* (Beyene et al. 2011), and Zewdu (2004) isolated *Salmonella* Newport from stool sample. Therefore, detection of these two serovars at the highest prevalence indicate public health concern in the study area as these organisms may reach to the consumer along the production chain, which become more serious in Bahir Dar in particular and in Ethiopia in general as the tradition of consuming raw or undercooked meat is common. *Salmonella* Heidelberg was previously isolated from camels (Molla et al. 2004) and sheep (Molla et al. 2006). As to the authors' knowledge, *Salmonella* Heidelberg was not isolated from cattle and *Salmonella* Haifa was not previously reported in Ethiopia; therefore, the detection of these two serovars from cattle in the current study indicates change in distribution to different population (the case of *Salmonella* Heidelberg) and importance (the case of *Salmonella* Haifa) of *Salmonella* serovars. The similarities of serovars between the different sample types might be associated with potential contamination during the slaughtering process either from the animals themselves, from the slaughterhouse personnel or from other common sources. According to Bouchrif et al. (2009), the leading source of contamination of carcasses by *Salmonella* is the evisceration step at the slaughterhouse.

## Antimicrobial resistance patterns

In the current study, 11 (39.3%) isolates were resistant to one or more of the tested antimicrobials. Four of the 28 (14.3%) isolates were MAR which was lower than the

**Table 2** The number and distribution of *Salmonella* serovars isolated from cattle slaughtered at Bahir Dar abattoir by animal and sample type

| No. of animals | Status of *Salmonella* | | | | Number of isolates |
|---|---|---|---|---|---|
| | Liver | MLN | IC | CS | |
| 1 | – | – | *Salmonella* Newport | Untypable | 2 |
| 2 | – | *Salmonella* Infantis | *Salmonella* Newport | *Salmonella* Infantis | 3 |
| 3 | – | *Salmonella* Typhimurium | – | *Salmonella* Typhimurium | 2 |
| 4 | – | – | *Salmonella* Infantis | *Salmonella* Infantis | 2 |
| 5 | – | – | *Salmonella* Typhimurium | *Salmonella* Typhimurium | 2 |
| 6 | *Salmonella* Typhimurium | *Salmonella* Typhimurium | *Salmonella* Newport | – | 3 |
| 7 | – | – | *Salmonella* Haifa | *Salmonella* Haifa | 2 |
| 8 | – | – | Untypable | *Salmonella* Newport | 2 |
| 9 | *Salmonella* Newport | *Salmonella* Newport | – | – | 2 |
| 10 | – | Untypable | *Salmonella* Heidelberg | *Salmonella* Heidelberg | 3 |
| 11 | – | *Salmonella* Haifa | *Salmonella* Mishmarhaemek | – | 2 |
| 12 | – | – | Untypable | *Salmonella* Mishmarhaemek | 2 |
| 13 | – | – | *Salmonella* Infantis | – | 1 |
| Total | 2 | 6 | 11 | 9 | |

**Table 3** Isolated serovars and their antimicrobial resistance patterns based on sample types

| Sample type | Serovar | Number of serovars | | Antimicrobial resistance pattern |
|---|---|---|---|---|
| | | Tested | resistant | |
| Liver | *Salmonella* Typhimurium | 1 | – | – |
| | *Salmonella* Mishmarhaemek | 1 | – | – |
| MLN | *Salmonella* Typhimurium | 2 | 1 | Chl, Str, Tet |
| | *Salmonella* Newport | 1 | 1 | Str |
| | *Salmonella* Infantis | 1 | – | – |
| | *Salmonella* Haifa | 1 | 1 | Amp, Str, Tet |
| | Untypable | 1 | 1 | Str, Tet |
| IC | *Salmonella* Typhimurium | 1 | – | – |
| | *Salmonella* Newport | 3 | 2 | Str |
| | *Salmonella* Infantis | 2 | – | – |
| | *Salmonella* Haifa | 1 | 1 | Amp, Str, Tet |
| | *Salmonella* Heidelberg | 2 | 2 | Str |
| | *Salmonella* Mishmarhaemek | 1 | – | – |
| | Untypable | 1 | – | – |
| CS | *Salmonella* Typhimurium | 2 | – | – |
| | *Salmonella* Newport | 2 | 1 | Str |
| | *Salmonella* Infantis | 2 | – | – |
| | *Salmonella* Haifa | 1 | 1 | Str |
| | Untypable | 2 | – | – |
| | Total | 28 | 11 | |

*MLN* mesenteric lymph node, *IC* intestinal content, *CS* carcass swab

reports of Molla et al. (2006) and Aragaw et al. (2007) in Ethiopia. The difference in the prevalence of multiple antimicrobial resistance *Salmonella* isolates might be associated with the difference in the use of antimicrobials both in human and public health in the different study areas. In Addis Ababa and central Ethiopia, drugs were easily available without prescription and indiscriminate use of antimicrobials were common (Molla et al. 2006). In Japan, Ishihara et al. (2009) reported that antimicrobial-resistant *Salmonella* have been isolated from poultry, swine, and cattle and present a growing concern to the public health.

Four types of antimicrobial resistance patterns including Str, StrTet, AmpStrTet, and ChlStrTet were detected among the different serovars. In consistent with Sibhat et al. (2009), the highest level of resistance (66.7%) was seen in *Salmonella* Newport to streptomycin. All serovars of *Salmonella* Heidelberg and *Salmonella* Haifa were resistant to streptomycin. Two isolates of *Salmonella* Haifa were resistant to ampicillin, streptomycin, and tetracycline. Previously, MAR up to ten antimicrobials of *Salmonella* Typhimurium was reported (Molla et al. 2006; Aragaw et al. 2007). The differences in the level of resistance of the isolated serovars from previous studies might be associated with differences in the frequency and type of antimicrobials used in the study areas. Alexander et al. (2009) described that increased frequency of antimicrobial-resistant *Salmonella* isolated from humans has led to concern about the contribution animal production systems have played in the emergence and spread of antimicrobial-resistant *Salmonella*. Antimicrobial resistance is a global problem in general (Acha and Szyfres 2001), but it might be more severe in Ethiopia where there is lack of antimicrobial resistance assessments of *Salmonella* and lack of stringent regulations but there is easy access of antimicrobials for purchase of people without prescription and incomplete treatment courses as the result of patient noncompliance.

In summary, six different *Salmonella* serovars including *Salmonella* Typhimurium, *Salmonella* Newport, *Salmonella* Infantis, *Salmonella* Haifa, *Salmonella* Heidelberg, and *Salmonella* Mishmarhaemek were detected, and all tested isolates were susceptible to the antimicrobial effects of gentamycin, norfloxacin, and trimethoprim. Antimicrobial resistance was observed mainly to streptomycin followed by tetracycline and ampicillin. Both single and multiple antimicrobial resistance patterns were observed, which is of special concern in Ethiopia where use of antimicrobials has problems. In animals, there is treatment restriction because of inadequate drug alternatives; therefore, limited drugs are frequently used for treatment. In humans, there are drug alternatives. However, people have easy access to various antimicrobials and can purchase without prescription, and incomplete treatment courses due to patient noncompliance are common practices. Results of this study suggest the need to maintain hygiene during slaughtering operations at

the slaughterhouse and consumer awareness on proper cooking of meat and meat products before consumption and antimicrobial resistance. Further detailed studies involving different abattoirs, animal products, food items, and animals on different settings were recommended in the study area.

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