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Antimicrobial Resistance Profile of *Salmonella* Species Isolated from Slaughtered Cattle Carcass and Slaughter House Environment in Dessie Municipality Abattoir, Ethiopia

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ABSTRACT

Food animals harbor a wide range of *Salmonella* and so act as sources of contamination. Salmonellosis is more aggravated by the ever increasing rate of antimicrobial resistance species in food animals. This study was aimed at investigating antimicrobial susceptibility of *Salmonella* species from slaughtered cattle carcass and slaughter house environment in Dessie municipality abattoir from December 2014 to August 2015. A total of 384 samples were collected, from 128 slaughtered cattle carcass (128 samples) and 256 environmental samples (128 from eviscerating knives and 128 from eviscerating hand) and examined for the presence of *Salmonella*. Out of 384 samples collected, 19 (4.95%) showed positive results for *Salmonella* species. From these, 8 (42.11%) of the isolates were *Salmonella* group-A, 7 (36.84%) *Salmonella arizana*e and the remaining 4 (21.05%) *Salmonella* isolates were *Salmonella* Typhi. All *Salmonella* isolates were tested for their susceptibility to 9 selected antimicrobial agents by the Kirby-Bauer disc-diffusion method. *Salmonella* isolates in this study were highly resistant to cefoxitin 13 (68.4%) followed by ampicillin 12 (63.2%). About 94.7% of the isolated *Salmonella* species were resistant to one or more antibiotic agents. However, all isolates (100%) were sensitive to ciprofloxacin, co-trimoxazole and gentamicin. Consumer aware on proper cooking of meat and meat products before consumption, and restricting, discriminate and appropriate use of antibiotics in the food animal industry were solutions to reduce the prevalence of antimicrobial resistance *Salmonella* in slaughtered cattle carcass and slaughter house environment.

Keywords: Antimicrobial susceptibility, Carcass, Eviscerating environment, *Salmonella*.

INTRODUCTION

The extensive use of antimicrobials in human and animals has led to an increase in bacterial multidrug resistant among several bacterial strains (Alambedji et al., 2006). The high prevalence and dissemination of multidrug resistant (MDR) *Salmonella* have become a growing public health concern. Of particular significance is the increasing number of *Salmonella* isolates that are resistant to clinically important antimicrobial agents such as fluoroquinolones and third-generation cephalosporins, which are used for the treatment of life threatening disease conditions in humans (Berhane et al., 2011; Abebe et al., 2004). Antimicrobial resistant *Salmonella* are increasing due to the use of antimicrobial agents in food animals (Addis et al., 2011). The increase in *Salmonella* resistance to the commonly used antimicrobials both in the public health and veterinary sectors is one of the major threats of health care worldwide (Molla et al., 2003). Cattle have been implicated as a source of human infection with antimicrobial resistant *Salmonella* through direct contact with livestock and through the isolation of antimicrobial resistant *Salmonella* from raw milk, cheddar cheese, and hamburger meat (Addis et al., 2011).

In Ethiopia, food animal consumption is a potential cause for antimicrobial resistant *Salmonella* illnesses besides, the common factors such as overcrowding, poverty, inadequate sanitary conditions, and poor personal hygiene. In addition to food items such as minced beef, mutton and pork samples obtained from retail supermarkets and slaughter house, supermarket and slaughterhouse personnel arealso a victim of *Salmonella* contamination (Zewdu, 2004; Ejeta et al., 2004). There is no any published and accessible information related to it in the region. In addition to this the antimicrobial susceptibility of *Salmonella* species is variable from time to time. So, updated information on their resistance

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patterns is very important for proper selection and use of antimicrobial agents in a setting. Therefore, the study focused on investigating the antimicrobial susceptibility of *Salmonella* species from slaughtered cattle and slaughterhouse environment in Dessie town from December 2014 to August 2015. The study would contribute to realize the current status of commonly used antibiotics and finally to generate information on the possible antibiotics to treat salmonellosis for physicians, concerned governmental and nongovernmental sectors, and for all stakeholders.

MATERIALS AND METHODS

Study period and sample collection:

The study was conducted from December 2014 to August 2015 to investigate antimicrobial susceptibility of *Salmonella* species isolated from slaughtered cattle carcass and slaughterhouse environment in Dessie Municipality Abattoir. A total of 384 samples were collected from128 slaughtered cattle carcass and256 environmental samples (128 from eviscerating knives and 128 from eviscerating hand). Cattle carcass surface and abattoir environment were swabbed by wiping with sterile cotton swabs on each sampling site five times in both vertical and horizontal directions (Gideon et al., 2010) for 30 seconds using sterile surgical glove. Cotton swabs were moistening in 10 ml sterile buffered peptone water (Cedex, France). The swab samples were transported from the site of collection to Wollo University biology laboratory using an ice-box and analyzed for the presence of *Salmonella* within one hour of collection.

Isolation and identification of *Salmonella*:

Swab samples were homogenized by shaking manually with 10 ml sterile peptone water (Merck, Darmstadt) and transferred to 10 ml of Selenite Cystine broth (Merck, Darmstadt) prior to sub-culturing onto Xylose-Lysine Deoxycholate (XLD) agar (Oxoid, England). The plates were incubated under aerobic atmosphere at 37^{o}C for 24 hours. Pink to red colonies with/without black center on Xylose-Lysine Deoxycholate agar were picked and streaked onto nutrient agar (Oxoid, England) for purification. Purified colonies were used as far as possible by inoculated into Tryptone Soy broth (Oxoid, England) to maximize the process of identification. Carefully selected colonies were picked and sub-culturing onto Tryptic Soy agar. A loop full of presumptive *Salmonella* colonies inoculums was taken from the Tryptone Soy agar culture and was used to carry out biochemical tests using Klingler iron agar, Sulfide-Indole-Motility (SIM), Lysine deoxycholate (Lysine Iron agar), urea agar and Simmons citrate agar media to obtain the true picture of biochemical tests (Cheesbrough, 2006).

Antimicrobial susceptibility tests:

All The Kirby-Bauer disc-diffusion test, which conforms to the recommended standard of the Clinical and Laboratory Standards Institute (CLSI, 2014), was used. Antimicrobial susceptibility testing of *Salmonella* isolates was done on the Mueller-Hinton agar (Oxoid, England) on the following antibiotics discs with their corresponding concentration; ampicillin (AMP, 10µg), tetracycline (TE, 30µg), cotrimoxazole (SXT, 25µg), gentamicin (CN, 10µg), chloramphenicol (C, 10µg), norfloxacin (NOR, 10µg), ciprofloxacin (CIP, 5µg), cefoxitin (FOX, 30µg), and nalidixic acid (NA, 30µg) (Oxoid). Morphologically identical 4-6 bacterial colonies were suspended in 5 ml Tryptone Soy broth and incubated for 4 hours at 37^{o}C. The bacterial suspension was compared with 0.5 McFarland turbidity standards. After adjusting the turbidity of the inoculum a sterile cotton swab was dipped into the suspension. The swab was streaked over the dried surface of Muller-Hinton agar. The nine commercially available anti-microbial disks were placed on the plate at least 15 mm apart and from the edge of the plates. After the disks were placed, the plates were incubated at 37 ºC for 18-24 hours. Following incubation, clear zones produced by antimicrobial inhibition of bacterial growth were measured to the nearest millimeter using metal calipers. The diameters of zones of inhibition were compared with recorded diameters of the control organism to interpret the strains as susceptible, intermediate or resistant. Antibiotics were chosen based on the prescription practices for *Salmonella* in this locality and from the literature. An isolate was defined as resistant if it was resistant to one or more of the antimicrobial agents tested whereas multiple resistances were define as resistance to 2 or more antimicrobial agents (CLSI, 2014). *Escherichia coli* ATCC 25922 was used as quality control.

RESULTS

Prevalence and distribution of *Salmonella* species:

Out of 384 cattle carcass and slaughterhouse environment swab samples collected, 19 (14.8%) showed positive results for *Salmonella* species (Table 1). Of 19 positive carcass, evisceration hand and knife examined, 9 (7.0%) were on the cattle carcass, 6 (4.7%) were on the evisceration hand and 4 (3.1%) were on the eviscerating knife. Relatively high numbers of *Salmonella* were detected in cattle carcass than the evisceration hand and eviscerating knife. From the total 19 *Salmonella* isolates, 8 (42.1%), 7 (36.8%) and 4 (21.0%) were *Salmonella* group-A, *arizana*e and Typhi, respectively, which were found on the three

sample types, even though itwas vary in number in sample type (Table 2).

Table 1: Isolation rate of *Salmonella* in cattle carcass and slaughter house environment, Dessie Municipal Abattoir, 2015.

Sample type	No. of Samples	No (%)
Cattle carcass	128	9 (7.0)
Eviscerations Hand	128	6 (4.7)
Eviscerations Knife	128	4 (3.1)
Total	384	19 (14.8)

Table 2: Number and distribution of *Salmonella* species from slaughtered cattle carcass and slaughterhouse environment, Dessie Municipal Abattoir, 2015.

Subspecies	Number (%) of isolates
S. group-A	8 (42.1)
S. arizanae	7 (36.8)
S. Typhi	4 (21.0)
Total	19 (100)

Antimicrobial sensitivity testing:

The antimicrobial susceptibility pattern of *Salmonella* isolates from cattle carcass, evisceration hand and eviscerating knife is shown in Table 3. The result documented that, all the isolated *Salmonella* species tested showed susceptibility to gentamicin, co-trimoxazole, and ciprofloxacin. On the other hand, 15 (78.9%) of *Salmonella* species isolated were susceptible to norfloxacin and chloramphenicol.

Antimicrobial drug resistant *Salmonella* isolates are presented in (Table 4). The highest resistance was documented for cefoxitin, 13 (68.4), and ampicillin, 12 (63.2%). The isolated *Salmonella* species also showed resistance against tetracycline 10 (52.6%) and nalidixic acid 3 (13.0%). About 94.7% of the isolated *Salmonella* species were resistant to one or more antibiotic agents. Only 1 (5.2%) *Salmonella* isolates were susceptible to all of the nine tested antibiotics.

Table 3: Antibiotic susceptibility patterns of *Salmonella* species isolates (N = 19)

Antibiotics	Susceptibility patterns		
	R No. (%)	I No. (%)	S No. (%)
Ampicillin	12 (63.2)	4 (21.0)	3 (15.8)
Cefoxitin	13 (68.4)	2 (10.5)	4 (21.1)
Chloramphonicol	0 (0.00)	4 (21.1)	15 (78.9)
Ciprofloxacin	0 (0.00)	0 (0.00)	1 9 (100)
Co-trimoxazole	0 (0.00)	0 (0.00)	19 (100)
Gentamicin	0 (0.00)	0 (0.00)	19 (100)
Nalidixic acid	3 (15.9)	7 (36.8)	9 (47.3)
Norfloxacin	1 (5.3)	3 (15.8)	15 (78.9)
Tetracycline	10 (52.6)	4 (21.1)	5 (26.3)

R – Resistant; I – Intermediate; S – sensitive;
P-value < 0.001

Table 4: Antimicrobial drug resistant of *Salmonella* isolates

Number of drug resisted	Resistant *Salmonella* isolates	
	No.	% (Percent)
R0	1	5.2
R1	6	31.6
R2	6	31.6
R3	4	21.1
R4	1	5.2
R5	1	5.2

R0 = susceptible to all; R1, R2, R3, R4 and R5, resistant to 1, 2, 3, 4 and 5 antimicrobials tested respectively.

Table 5: Multidrug resistance pattern of *Salmonella* isolates

Resistance Pattern	*Salmonella* isolates No. (%)
Resistance to two antibiotics	
AMP, FOX	10 (52.6)
TE, FOX	7 (36.8)
AMP, TE	6 (31.6)
TE, NOR	1 (5.3)
NOR, AMP	1 (5.3)
FOX, NOR	1 (5.3)
NOR, NA	1(5.3)
NA, TE	3 (15.8)
NA, FOX	2 (10.5)
NA, AMP	2 (10.5)
Resistance to three antibiotics	
TE, FOX, AMP	6 (31.6)
TE, NOR, FOX	1 (5.3)
TE, AMP, NOR	1 (5.3)
AMP, FOX, NOR	1 (5.3)
NA, TE, FOX	2 (10.5)
NA, TE, AMP	2 (10.5)
NA, AMP, FOX	2 (10.5)
NOR, TE, NA	1 (5.3)
NOR, NA, FOX	1 (5.3)
NOR, NA, AMP	1 (5.3)
Resistance to four antibiotics	
TE, NA, AMP, FOX	2 (10.5)
TE, NA, NOR, AMP	1 (5.3)
TE, NA, NOR, FOX	1 (5.3)
Resistance to five antibiotics	
AMP, FOX, NA, NOR, TE	1 (5.3)

Note, AMP = ampicillin; FOX = cefoxitin; NA = nalidixic acid; TE = tetracycline; NOR = norfloxacin.

The *Salmonella* isolates from slaughtered cattle carcass and slaughter house environment showed multiple antimicrobial resistance patterns ranging from two to five antibiotics. Multidrug resistance pattern of *Salmonella* isolates are presented in (Table 5).The highest multiple drug resistance *Salmonella* species were seen against two commonly used antimicrobial agents, ampicillin and cefoxitin. Only10 (52.6%) of the isolated *Salmonella* species were resistant against ampicillin and cefoxitin. This multiple drug resistance of *Salmonella* isolates was also recorded against TE, FOX and AMP, 31.6%. In the present study, 10.5% of the *Salmonella* isolates were found to be resistant to four antibiotics (AMP, FOX, TE, and NA) and 5.3% of *Salmonella* isolates were resistance to five antibiotics (AMP, FOX, NA, NOR, and TE). In general, out of the total 19 *Salmonella* isolates, 12 (63.1%) were showed multidrug-resistant.

DISCUSSION

Prevalence and distribution of *Salmonella* species:

The proportion between samples type in which *Salmonella* were detected in the same day is relatively higher in cattle carcass and lower in eviscerating knife. According to the study conducted by Teklu & Negussie (2011) in Mojo, Ethiopia, carcass samples are more contaminated than samples taken from the environment. The reason might be cattle carcass contamination were the overall effect of the slaughterhouse environment and the cattle itself. The result of this study is also in line with the study documented in Jimma, Ethiopia which revealed that the prevalence of *Salmonella* in meats and faeces samples collected from the investigated Abattoir was only 9 (4.4%) indicating the relatively better hygienic practice during processing and handling the meat at the Abattoirs (Dabassa & Bacha, 2012). The prevalence of *Salmonella* group-A and *Salmonella arizana*e in the present study are lower but the prevalence of *Salmonella* Typhi is higher than with the study conducted in Bahir Dar, Ethiopia (47.8%, 39.1% and 13.1%, respectively) (Muluneh & Kibret, 2015). This indicates that the carcass as well as the environment were exposed to human contact. The distribution of *Salmonella* species among cattle varies greatly over time, and differs among geographic regions, age groups, clinical manifestation, and production systems (Hoelzer et al., 2011). In this study the existence of *Salmonella* groups-A, 8 (42.11%) and *Salmonella* Tyhpi, 4 (21.05%) indicate that the contamination is of human origin and result of poor personal hygiene during handling and processing of the meat products (Mrema et al., 2006; Tassew et al., 2010).

Antimicrobial sensitivity testing:

The susceptibility of *Salmonella* species to fluoroquinolones and gentamicin is in line with the study conducted in different parts of Ethiopia: Jimma (Dabassa &Bacha, 2012), Bahir Dar (Alemu & Molla, 2011), Harrer (Reda et al., 2011). Similarly, the susceptibility of *Salmonella* species to chloramphenicol, ciprofloxacin and gentamicin is in agreement with the study conducted in Jimma, Ethiopia (Dabassa &Bacha, 2012), Harrer, Ethiopia (Reda et al., 2011), in Tehran (Dallal et al., 2009) and in Bhutan (Narapati, 2007). This would probably be due to the fact that the drugs are relatively expensive, limited availability and newly introduced, compared to the other commonly used antibiotics. This showed that reduction of their frequent utilization in veterinary practice or public health practices **(**Alambedji et al., 2006). Thus, these antibiotics can serve as choice of drugs for salmonellosis in the study area which are also indicated by WHO (WHO, 2005). In contrast to this result, the studies conducted in different parts of Ethiopia reported that, fluoroquinolones (ciprofloxacin and norfloxacin) and gentamicin resistant *Salmonella* isolates have emerged at this time (Reda et al., 2011) in Harrer and except gentamicin (Molla et al., 2003) in Addis Ababa. Similarly the study conducted in other countries reported that, emerging resistance of *Salmonella* to new and powerful antibiotics (Emmanuel et al., 2011; Rajic et al., 2004) and the resistance of the above antibiotic also seen in Amaigbo, Nigeria (Sarah & Enwuru, 2014). The study documented in Nigeria revealed the majority of the isolate resistance to co-trimoxazole (Alao et al., 2012). Increasing of resistance to antimicrobial agents that are important in the treatment of human diseases, such as fluoroquinolones and gentamicin for the treatment of *Salmonella* infections, has significant public health implications (Anderson et al., 2003).

The development of resistance toampicillin is in line with the study conducted in different parts of Ethiopia: Bahir Dar (Alemu &Molla, 2011), Gonder (Garedew et al., 2015), Addis Ababa (Addis et al., 2011), and Harrer (Reda et al., 2011). The current result is higher than the studies conducted in other localities revealed that, only 44.3% of *Salmonella* were resistant to ampicillin (Chotinun et al., 2014). This might be due to the indiscriminate and misuse of antibiotics in animal and human creates a reservoir of resistant *Salmonella* in the animal that could infect humans through the food chain and the abuse of antibiotics in human medicine may instead be largely responsible for the increase in antibiotic resistance (Nawaz et al., 2001) and also the present finding stats that it is necessary prudent use of antimicrobial drugs is urgently needed if *Salmonella* contamination is to be reduced. The

resistance of tetracycline in this study is near to the study conducted in Nairobi, Kenya (34.2%) (Chotinun et al., 2014) and very low to compeer with the study conducted in Harer, Ethiopia (100%) (Ferede et al., 2015) and high compeer with the study presented in Gonder, Ethiopia (32.1%) (Garedew et al., 2015). Tetracycline and nalidixic acid resistance is in agreement with studies in Berlin, Germany (Ellerbroek et al., 2010), in Namibia (Renatus et al., 2012) and in Tehran (Dallal et al., 2009). On the other hand the resistance of cefoxitin in this study is near with the study conducted in USA (Dargatz et al., 2003) but study conducted in Canada showed that no resistance of *Salmonella* was observed in cefoxitin (Rajic et al., 2004). Similarly, resistance of *Salmonella* isolates to ampicillin, nalidixic acid and tetracycline are supported by study conducted in Ethiopia (Dabassa & Bacha, 2012). And also tetracycline had high resistance (81%) in the study conducted in Amaigbo, Nigeria (Sarah & Enwuru., 2014) compeer to the current study. This increased resistance to antibiotics might be due to the unwise use of antibiotics in many developing countries such as Ethiopia, which would have lead to an increased antibiotic resistance and in turn reduced therapeutic efficacy in these countries (Asrat, 2008). Results of this study also indicated that high level of carcass contamination with antimicrobial resistant *Salmonella* subspecies which could pose public health risk; suggests need for hygienic slaughtering operations and proper cooking of meat before consumption.

About 94.7% of the isolated *Salmonella* species were resistant to one or more antibiotic agents. This result is near with a study conducted in Addis Ababa, Ethiopia (Addis et al., 2011), which reported that about 83% of the isolated *Salmonella* species were resistant to two or more antibiotics, in Gonder, Ethiopia, (75.5 %) isolates of *Salmonella* showed resistance to two or more antimicrobial agents tested (Garedew et al., 2015).On the other hand opposite to this study, the study conducted in Bahir Dar, 39.3% of *Salmonella* isolates were resistant to one or more of the commonly used antimicrobial (Alemu & Molla, 2011). This result showed a lower rate of resistant *Salmonella* isolates in the same country. This might be due to a difference in resistance rate of *Salmonella* isolates from time to time. Studies in other countries like Kenya, United Arab Emirates, Maryland and Namibia, antimicrobial resistance to one or more antimicrobials were found in 35.7%, 16%, 21% and 19.7% of the isolates, respectively (Gideon et al., 2010; Li et al., 2012; Munch et al., 2012; Renatus et al., 2012). The studies in Kenya, United Arab Emirates, Maryland and Namibia also showed a lower rate of resistant *Salmonella* isolates than in the present study. This might be due to a difference in resistance rate of *Salmonella* isolates from place to place and from time to time. Only 1 (5.2%) *Salmonella* isolates out of the total 19 *Salmonella* isolates tested were susceptible to all of the nine tested antibiotics but study conducted in Jimma documented that, no isolates were susceptible to all of the antibiotics (Dabassa & Bacha, 2012) and 6 (31.6%) *Salmonella* isolate was resistant to one tested antibiotic. The study documented in other locality showed that 68.4% of the *Salmonella* species were resistant to at least one antimicrobial (Chotinun et al., 2014). The high level of antibiotic resistance of *Salmonella* isolates in the present study showed the possible significance of cattle carcass and slaughter house environment a source of multiple antimicrobial resistant *Salmonella* for human infections and suggests more restrictions on the irrational use of antibiotics (Abdellah et al., 2009).

Over all out of the total 19 *Salmonella* isolates, 12 (63.1%) were multidrug-resistant. Relatively low rate of multidrug resistance *Salmonella* isolates were seen in other studies in Addis Ababa, Ethiopia ranging two or more antibiotics than the current study (Addis et al., 2011). The study conducted in Gonder, report that 28.3 % of the isolates were multidrug-resistant (Garedew et al., 2015) and in other area like Korea (Kim et al., 2011). The prevalence of *Salmonella* strains resistant to more than one antibiotic may be due to the comprehensive use of antibiotics included in feeds as growth promoters and due to the wide spread use of antibiotics in animal food industries (Khan et al., 2010). The indiscriminate and inappropriate use of antibiotics in outpatient clinics, hospitalized patients and in the food industry is the single largest factor leading to multidrug resistance (Alanis, 2005). A high rate of multidrug resistance exists in the present study. This showed that, in this locality the presence of *Salmonella* resistant to antimicrobial drugs is common in cattle carcass slaughter house environment. The multidrug resistance of the present study is comparable with the study conducted in Dakar Senegal (Alambedji et al., 2006) and in Meknes, Morocco (Abdellah et al., 2009) and high rate of multidrug resistance documented in Ethiopia (83%) (Ferede et al., 2015). However, low rate of multidrug resistance *Salmonella* isolates were recorded in Ethiopia (Alemu & Molla, 2011) and in different studies like Sudan (Fadlalla et al., 2012),United Arab Emirates (Munch et al., 2012), in Bhutan (Narapati, 2007), in USA (Dargatz et al., 2003), in USA (Fluckey et al., 2007) and in Tehran (Dallal et al., 2009). This increased multidrug resistance in the present study compeer to others done elsewhere might be due to lack of restricting the use of antimicrobial agents in food animals, designation of multidrug-resistant *Salmonella* as an adulterant in ground beef, and improving the mechanisms for

product trace-back investigations (Elizabeth et al., 2006).

In conclusion, the result suggests that both single and multiple antimicrobial resistance patterns to the commonly used antimicrobials in the veterinary and public health set up 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; this practice leads resistance to limited antibiotics. Restricting the use of antimicrobial agents in food animals, designation of multidrug-resistant *Salmonella* as an adulterant in ground beef, improving the mechanisms for product trace-back investigations and wise and discriminate use of antimicrobials should be practiced to combat the ever increasing situation of antimicrobial resistance.

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