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Prevalence and antimicrobial susceptibility profile of *Salmonella* species from ready-to-eat foods from catering establishments in Jigjiga City, Ethiopia

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Food-borne pathogens are the leading cause of morbidity and mortality in developing countries. Changes in eating habits, mass catering, unsafe food storage conditions and poor hygiene practices are major contributing factors to food associated illnesses. In Ethiopia, the widespread habit of ready-to-eat food consumption is potential cause of food borne illnesses. The present study aimed at investigating the prevalence and antimicrobial susceptibility profile of *Salmonella* species from ready-to-eat foods from catering establishments in Jigjiga City and determining susceptibility pattern of bacterial isolates. This study aimed to assess the ready-to-eat foods *Salmonella* prevalence and antimicrobial resistance pattern of the isolates. In total, various food samples were collected from 120 hotels and processed to detect *Salmonella* presence. The ready-to-eat food samples were collected and assessed bacteriologically for *Salmonella* strains. A total of 120 food samples were bacteriologically evaluated. After confirmation, 25 samples (20.8%) were positive to *Salmonella*. A total of 5 drug resistance patterns were detected among *Salmonella* isolates. Out of the 25 isolates 10 (40%) were resistant to three antibiotics, 8 (28%) of the isolates were resistant to 4 of the antibiotics tested, whereas 5 (20%) were resistant to two antibiotics, only 1 (4%) isolate was resistant to 1 antibiotic but 1 (4%) isolate was resistant to five antibiotics. The lack of public sanitary facilities can be another hurdle to keep the desirable hands hygiene of the vendors. In this study, it is reported that severity of the current scenario among the hotel worker hygiene and they are the unknowingly playing role in spread of diseases like Salmonellosis.

Key words: Food Establishments, ready-to-eat foods, *Salmonella.*

INTRODUCTION

Foodborne outbreaks caused by *Salmonella* represent a major public health problem worldwide, and developing countries are affected by a wide range of foodborne diseases (Zeru and Kumie, 2007). These low-income countries face the highest burden of diarrheal and other food-borne disease associated with the consumption of contaminated food (Zeru and Kumie, 2007). *Salmonella* are among the major disease bacteria in humans as well as in animals. *Salmonella* species are leading causes of acute gastroenteritis in several countries

and salmonellosis remains an important public health problem worldwide, particularly in the developing countries (Rotimi et al., 2008). Salmonellosis is the most common food borne disease in both developing and developed countries, although incidence rates vary according to the country (Stevens et al., 2006). The fecal wastes from infected animals and humans are important sources of bacterial contamination of the environment and the food chain (Ponce et al., 2008).

Antimicrobial-resistant *Salmonella* are increasing due to the use of antimicrobial agents in food at sub-therapeutic level or prophylactic doses which may promote on-farm selection of antimicrobial resistant strains and markedly increase the human health risks associated with consumption of contaminated food products (Zewdu and Cornelius, 2009). Food borne illness associated with the consumption of foods has been reported in several places in Ethiopia and elsewhere (Estrada-Garcia et al., 2004; Chumber et al., 2007; Ghosh et al., 2007). Antimicrobial use in animal production systems has long been suspected to be the cause of emergence and dissemination of antimicrobial resistant *Salmonella* (Alexander et al., 2009).

Different studies conducted in Ethiopia indicated considerable prevalence of *Salmonella* both in ready to eat foods and food handlers (Molla et al., 2003). So, the aim of this study was to determine the prevalence and antimicrobial susceptibility pattern of *Salmonella* isolates from ready-to-eat foods from Jigjiga City food establishments.

MATERIALS AND METHODS

Description of the study area and period

This study was conducted in Jigjiga City, located 650 km South East of Addis Ababa. The study was conducted from March to October, 2015 and laboratory activities were carried out at veterinary medicine microbiology laboratory, Jigjiga University. The study samples were collected from different food establishments of the city. Sample size was determined from 256 food establishments using prevalence rate of 50%, there was no other baseline to determine the prevalence at 5% level of significance and the following formula was employed (Yemane, 1967):

$$n = \frac{N Z^2_{a/2} P (1-P)}{d^2 (N-1) + Z^2_{a/2} P (1-P)}$$

Based on the above formula, the calculated sample size was 120. A total of 120 food establishments and 6 food types were randomly selected and included in this study. All the samples including 20 kikil, 20 mahberawi, 20 beyayinet, 20 tibs, 20 minchet and 20 keywet were collected for the detection of *Salmonella*. The ready-to-eat food specimens were collected in a clean sterile aluminum foil directly from the kitchen. Approximately 25 g of ready-to-eat food was collected in a sterile aluminum foil. The samples were transported using an ice box and analyzed at veterinary medicine microbiology laboratory, Jigjiga University.

Isolation and identification of *Salmonella*

The isolation and identification of *Salmonella* were performed using techniques recommended by International Organizations for Standardization (ISO-6579, 2000), and those supported by the Global Salmonella Surveillance (GSS) and National Health Services for Wales (NHS) (HPA, 2008). The isolation and identification involves three steps; To test for the presence of *Salmonella*, 25 mm^3 of each sample was aseptically transferred into sterile flask containing 225 ml buffered peptone water (BPW), homogenized for 5 min and then incubated at 37°C for 24 h for recovery and proliferation of cells which might be injured during processing or to make the number of target organisms grow to a detectable level. Following the BPW enrichment, the secondary enrichment broth namely the Rappaport Vassiliadis enrichment broth was used since the selective property of this broth lies in its ability to inhibit non-targeted microorganisms like Gram positive bacteria and coliforms and permits the rapid multiplication of *Salmonella*. After pre-enrichment in buffered peptone water, 1 ml of culture from the buffered peptone water was transferred into 10 ml of Rappaport Vassiliadis broth and was incubated at 43°C for 48 h. Solid media such as Salmonella–Shigella agar, xylose lysine desocholate (XLD) agar and Brilliant green modified agar were used for plating purpose. A loopful of culture from the Rappaport Vassiliadis broth were streaked onto each of the solid medium and incubated at 37°C for 18 h. Characteristic colonies from each selective agar were picked, further purified and tested biochemically.

All suspected non-lactose fermenting bacterial colonies, picked from Salmonella-Shigella (SS) agar, XLD agar or Brilliant green modified agar, were inoculated into the following biochemical tube for identification: triple sugar iron (TSI) agar, Simmon's citrate agar, sulphide indole motility (SIM) medium, lysine iron agar, urea agar, and fermentation of glucose, sucrose and mannitol.

Antimicrobial susceptibility testing

The antimicrobial susceptibility patterns of *Salmonella* spp. were carried out following the Kirby–Bauer disc diffusion method on Mueller-Hinton agar plates (Oxoid) as described by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) guidelines (NCCLS, 2002). Antimicrobial susceptibility testing for *Salmonella* spp was performed using the disk diffusion method at 37°C for 16-18 h and results were interpreted using the criteria of the National Committee for Clinical Laboratory Standards. *Escherichia coli* ATCC 25922 was used as a quality control organism for the criterion for selection of the antimicrobial agents was based on the availability and current use of these antibiotics for treating infectious antimicrobial susceptibility test (Hendriksen, 2002). The disease in health institution of Ethiopia. The following antibiotics were used with their respective concentration (in brackets) for

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Table 1. The prevalence of *Salmonella* from different food types at catering establishments.

Establishments type	Sample size	*Salmonella* positive	*Salmonella* positive (%)	P value
Beyayinet	20	6	30	
Kikil	20	5	25	
keywet	20	3	15	
Mahberawi	20	9	45	p= 0.023
Tibs	20	0	0	
Minchet	20	2	10	
Total	120	25	20.8	

Table 2. Antimicrobial resistance of *Salmonella* isolates by food establishment types (n=25).

Antimicrobial disc	Total number (%) isolates resistant from foods served at food establishments					
	Total isolate (n=25)	Beyayinet (n=6)	Kikil (n=5)	keywet (n=3)	Mahberawi (n=9)	Minchet (n=2)
AMP	25 (100)	6 (100)	5 (100)	3 (100)	9 (100)	2 (100)
STR	2 (8)	1 (16.7)	0 (0)	0 (0)	1 (11.1)	0 (0)
NOR	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
TET	7 (28)	2 (33.3)	1 (16.7)	1 (33.3)	3 (33.3)	0 (0)
KAN	16 (64)	4 (66.7)	3 (60)	2 (66.7)	6 (66.7)	1 (50)
GEN	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
CHL	6 (24)	2 (33.3)	1 (20)	0 (0)	2 (22.2)	0 (0)
CIP	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NAL	22 (88)	5 (83.3)	4 (80)	3 (100)	8 (88.9)	2 (100)

Salmonella spp. isolates: chloramphenicol (30 µg), ciprofloxacilin (15 µg), amoxacillin (15 µg), gentamicin (10 µg), erythromycin (20 µg), ampcillin (10 µg), co-trimaxazol (10 µg), doxacyclin (30 µg), ciprofloxacillin (30 µg) (Micromaster, India).

Data analysis

Data was analyzed using SPSS, version 17.0 computer soft-ware (SPSS 17.0 Command Syntax Reference. SPSS Inc., Chicago, 2004) and presented in tables. A significant difference was taken as significant at a $p \leq 0.05$.

Ethical consideration

The study was ethically approved by the Institutional Review Board of Jigjiga University, research and ethical clearance committee.

RESULTS

From a total of 120 samples examined, 25 (20.8%) meals from food establishments were found to be positive to *Salmonella* spp. (Table 1). Moreover, *Salmonella* spp. were isolated from 6 (30%) beyayinet, 3 (15%) keywet, 5 (25%) kikil and 9 (45%) of mahberawi, none of tibs and 2 (10%) of minchet. The frequencies of isolation of *Salmonella* spp. differed among the food types and it ranged from 0 (tibs) to 45% (mahberawi) (Table 1). Significant variation was observed in prevalence of *Salmonella* among food types (p= 0.023).

A total of 25 isolates (n=25) were tested against nine commonly used antimicrobials viz. ampicillin (10 µg), chloramphenicol (30 µg), gentamycin (10 µg), streptomycin (10 µg), kanamycin (30 µg), Nalidixic Acid (30 µg), Ciprofloxacin (5 µg), tetracycline (25 µg) and norfloxacin (10 µg) following NCCLS 2000 guidelines. The results of the antimicrobial sensitivity test are shown in Table 2.

Among all the antimicrobials tested, Ampicillin (100%) and Nalidixic Acid (88%) were the most resistant drugs by *Salmonella* isolates followed by kanamycin (64%), tetracycline (28%) and chloramphenicol (24%). norfloxacin (0%), gentamycin (0%) and ciprofloxacin (0%) showed maximum activity.

A total of 5 drug resistance patterns were detected among *Salmonella* isolates (Table 3). Out of the 25 isolates 10 (40%) were resistant to 3 antibiotics, 8 (28%) of the isolates were resistant to 4 of the antibiotics tested, whereas 5 (20%) were resistant to 2 antibiotics, only 1 (4%) isolate was resistant to 1 antibiotic but 1 (4%) isolate was resistant to 5 antibiotics (Table 3).

DISCUSSION

Salmonella was found in 20.8% of food samples in

Table 3. Multi drug resistance pattern of *Salmonella* spp. isolated from different food types of food establishments.

MDR patter	Resistance patter	Number of isolates	Percent
One	Amp	1	4
Two	Amp, Nal	3	12
	Amp, Str	2	8
Three	Amp, Kan, Nal	7	28
	Amp, Tet, Nal	1	4
	Amp, Chl, Nal	1	4
	Amp, Str, Chl	1	4
Four	Amp, Tet, Kan, Nal	1	4
	Amp, Tet, Kan, Nal	4	16
	Amp, Kan, Chl, Nal	3	12
Five	Amp, Tet, Kan, Chl, Nal	1	4

Amp = Ampicillin, Str = streptomycin, Nor = norfloxacin, Tet = tetracycline, Kan = kanamycin, Gen = gentamycin, Chl = chloramphenicol, Cip = ciprofloxacin, Nal = naldixic acid.

this study. This is a pathogenic micro-organism at very low doses of infection, and is transmitted from person to person but may also occur by consumption of contaminated water and foods including vegetables that have received little or no heat treatment. Food may become contaminated by infected food handlers who do not wash their hands with soap after using the toilet. Foods can also become contaminated if they are harvested from a field with sewage contamination in them and thus the need for caterers to ensure they buy the raw foods from reputable suppliers. In addition, frequent assessing of the raw food product supplier is encouraged.

Salmonella can also be transmitted by flies. Flies can breed in infected feces and then contaminate food and thus the need for caterers to have an elaborate pest control program. The prevalence of *Salmonella* in foods was too high, in contrast to the previous studies in Ethiopia. For instance, Akafete and Haileleul (2011) and Woldemariam et al. (2005) found that the prevalence of *Salmonella* from goat carcass swab was 8.3% at Modjo and 7.5% at Bishoftu, respectively. This difference could be due to differences in the hygienic and sanitary practices practiced in the food establishments at Jigjiga city.

Resistance to multiple antimicrobials (100%) which was observed in the current study was higher than other studies conducted in Ethiopia. There are reports which shows the multiple antibiotics resistance by *Salmonella*, for instance, Alemayehu et al. (2002), Endrias (2004) and Zelalem et al. (2011) reported 52, 23.5, 44.8 and 83.3%, respectively for the multidrug resistance of *Salmonella* isolated from food of animal sources, animals and humans, as well higher than reports from elsewhere (Stevens et al., 2006; Khaitsa et al., 2007; Al-Bahry et al., 2007; Elgroud et al., 2009; Fadlalla et al., 2012) on multidrug resistance of *Salmonella.* This difference could be because, drug-resistant *Salmonella* are increasing due to the use of antimicrobial agents in food animals at prophylactic doses which may promote on-farm selection of antimicrobial resistant strains and markedly increase the human health risks associated with consumption of contaminated food products as stated by Molla et al. (2003, 2006) and Zewdu and Cornelius (2009).

Zewdu and Cornelius (2009) reported that the isolates of *Salmonella* from food items and workers from Addis Ababa were resistant to the commonly used antibiotics including streptomycin, ampicillin, and tetracycline. Furthermore, Zelalem et al. (2011) also indicated resistance of *Salmonella* isolates to commonly used antimicrobials including ampicillin, streptomycin, nitrofurantoine, kanamycine and tetracycline, with resistance rate of 100, 66.7, 58.3 and 33.3%, respectively. Similarly, previous reports from South India (Suresh et al., 2006), from Nigeria (Akinyemia et al., 2005) and from Cameroon (Akoachere et al., 2009) indicated a similar 100, over 90 and 100%, respectively resistance to ampicillin. The result of the current research also showed resistance of *Salmonella* isolates to commonly used antimicrobials including ampicillin, nalidixic acid and kanamycin with resistance rate of 100, 88 and 64%, respectively. However, higher resistance rate than previous reports with the exception of ampicillin and resistance to further drugs as well as to nalidixic acid with resistance rate of 56.8% was observed in this result. This difference could be due to the increasing rate of inappropriate utilization of antibiotics which favors selection pressure that increased the advantage of maintaining resistance genes in bacteria (McGeer, 1998;

Mathew et al., 2007). It is as well recognized that recent resistance additions include resistance to trimethoprim. The continuing development of antibiotic resistance may lead to sufficient pressure ultimately to restrict the antibiotics available to the veterinary profession for animal treatment (Gracey et al., 1999). Moreover, this increase antibiotic resistance, in addition to public health problems, may lead to economic loss in the countries due to loss of exporting meat and animal products and cost of drug of choice to treat human and animals due to resistance development.

Ciprofloxacin showed a good antimicrobial activity against these *Salmonella* isolates. It was found that all the 25 (100%) isolates were susceptible to ciprofloxacin. This result was comparable to previous reports by Molla et al. (2006) from central part of Ethiopia among isolates of sheep and goat meat, Akinyemia et al. (2005) from Nigeria, from human isolates and Zelalem et al. (2011), isolates of *Salmonella* from dairy farms in Addis Ababa. The effectiveness of drugs like ciprofloxacin could be because they are not widely used in countries like Ethiopia and other African countries (Zelalem et al., 2011). In addition to this, effectiveness of this drug could be because it is not well distributed in all societies and not simply prescribed rather it is used as drug of choice in antibiotic resistant person. In addition to this, ciprofloxacin is not commonly used to treat animals in Ethiopia.

The currents study indicated the inevitability of a further research on the prevalence and antimicrobial susceptibility of *Salmonella*, by considering it as a potential food borne pathogen. Molecular characterization of the isolates with emphasis on resistant strains is also required to identify mechanisms of resistance. Moreover, careful and discreet use of antimicrobials in the health sectors is mandatory since high rate of antimicrobial resistant *Salmonella* isolates were identified.

Conflict of Interests

The authors have not declared any conflict of interests.

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