

***Shigella* Serogroups, Entero-Hemoragic *E. coli* and Their Antibiogram Pattern Among Food Handlers in Food-Handling Establishments in Southern Ethiopia**

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Abstract: Food-borne illnesses have a dramatic impact both in developing and developed countries. Food handling personnel take part in the transmission of pathogenic food born bacteria in the community. *Shigella* and *E. coli* 157:H7 are more significant and well-recognized foodborne pathogens for reasons of their severe consequences of all age groups, high antibiotic resistance and their low infectious dose. Accordingly, food-handlers employing in meal serving facilities could be potential sources of infections of these enteric bacterial pathogens. Community based cross-sectional study was carried out from July 2014 to June 2015 to assess the sero-group and antimicrobial resistance pattern of enteric bacterial pathogens in Wolaita Sodo town among 398 food-handlers working in selected food handling establishments. Pre-tested structured questionnaire was used to collect Socio-demographic characteristics and associated factors. Stool specimens were collected by a clean, dry, wide-mouthed container. Stool culture was done using differential, selective and enrichment medium. Analytical Profile Index 20E biochemical panel was used for identification and differentiation of members of enteric bacterial pathogens. Antibiotic susceptibility testing was done by single disk diffusion technique. Data entry and analysis were done using SPSS version 20. 11 *Shigella* species and 24 *E. coli* O157:H7 isolates were detected. *S. flexneri*, *S. sonnei*, *S. dysenteriae*, and *S. boydii* isolates were isolated. A significant proportion of Ampicillin and Amoxicillin were noticed for all enteric bacterial pathogens. Multidrug resistances prevalence of 72.7%, and 58.3% were observed for *Shigella*, and *E. coli* O157: H7 respectively. Raw meat eating habit, hand washing after toilet and hand washing after touching dirty materials have shown significant association with enteric bacterial pathogens prevalence. *Shigella* and *E. coli* species were identified from fecal specimen. Significant proportion of multidrug resistances was detected in *Shigella* and *E. coli* O157: H7 respectively. Thus screening of food handles is important in order to prevent the transmission of enteric bacterial pathogens and treatment needs to be based on accurate laboratory detection of etiologic agents to mitigate the spread of drug resistant strains.

Keywords: *E. coli* O157:H7, *Shigella*, Food Handlers, Antibiotics

1. Background

Sufficient and safe food is vital to the health and well-being of humans. However, contaminated food serves as vehicle to transmit food born bacteria from infected individuals to healthy person. Food born infections are common problems both in developed and developing countries while the severity of problem is varied in different

countries. It resulted in morbidity and mortality all over the world [1]. Food borne microbial pathogens averagely causes diarrhoeal diseases among 30% of people living in developed countries. However, it is serious among people living in developing countries; more than 2 million die on average [2].

Microbes like bacteria, viruses, and parasites are often

associated with food born diseases and more than 250 diseases have been identified yet [3]. Typhoid, cholera, hepatitis A, food poisoning and dysentery are among common infections associated with contaminated foods [1].

Enterobacteriaceae family usually present in the gastrointestinal tract of human being as a normal flora and genus of this family like, *Shigella* and *Escherichia* produce disease in human being when they get conducive environment [4].

Shigellosis, an acute invasive enteric infection caused by *Shigella* species, is also recognized as a major public health problem [5]. It is transmitted either through the feces of an infected person or through food or water contaminated by an infected person, with as few as 10 to 100 organisms capable of causing infection [6]. According to the Centers for Disease Control and Prevention (CDC) emerging infections program, *Shigella spp.* was the third most reported food-borne bacterial pathogen in 2002 [7].

Food handling personnel take part in the transmission of pathogenic food born bacteria in the community [8], [9]. Food handlers may be sources of organisms either during the course of gastrointestinal illness or during and after convalescence, when they no longer have symptoms. Several studies have indicated that various bacteria, survive on hands and surfaces for hours or even days after initial contact with the microorganisms [10].

All food establishments have a responsibility for ensuring customers are provided with safe food. In some cases, meals served at these establishments are implicated in foodborne disease outbreaks [11]. In Ethiopia provision of safe food should have been the primary focus in order to mitigate the spread of easily preventable food born bacteria while little attention has been given [8]. In Wolaita Sodo town, the increasing number of establishments such as hotels, restaurants and snack bars is an indication of a growing tendency to eat and drinking in such places. Therefore this study was designed to assess the sero-group of enteric bacterial pathogens, associated factors and their antimicrobial resistance pattern among food handlers working in Wolaita Sodo town Food handling establishments, Southern Ethiopia.

2. Methods and Materials

2.1. Study Area

The town is located at 380 Kms South of the capital Addis Ababa. The town is the largest city in Wolaita Zone, has 3 sub-cities. According the Central Statistical Authority, the projected total population of the town is 86,050. There were 68 meal serving facilities (Restaurants and Cafeteria) in the town during the study period.

2.2. Study Design and Period

A community based cross-sectional study was carried out among food-handlers working in randomly selected meal serving facilities in Wolaita Sodo town from July 2014 to June 2015.

2.3. Sample Size and Sampling Procedures

Sample size was determined to achieve 95% confidence interval. We assumed 50% of the proportion of food handles would be involved with a 5% of margin of error and 10% of non response rate. The source population was less than 10,000. Thus, correction formula was used to adjust and gave 218. Moreover, design effect 2 was taken and the final calculated sample size was 436.

2.4. Sampling Technique

A systematic random sampling technique was employed to select study participants. A list of all food establishments in each sub-city was obtained from the municipality. Respondent was included from every selected meal serving facilities proportional to the number of meal serving facilities present in the sub-cities.

2.5. Sample Collection and Analysis

Stool specimen along with Socio demographic variables were collected from study subjects by health care professional, laboratory technologist and sanitarian after getting written consent. Freshly passed stool samples were passed directly into a clean, dry, wide-mouthed container and transferred in to Cary Blair transport medium using the scoop until the level of liquid reaches the fill line and transported to Wolaita Sodo University Microbiology laboratory using cold box.

2.6. Laboratory Investigation

Each stool sample was streaked onto Hecktoen agar and pre-enriched in selenite broth at 37°C for 24 h. The pre-enrichment sample was streaked onto Hecktoen agar, and after incubation at 37 °C for another 24 h, the suspicious colonies were identified with biochemical test. Suspicious colonies were identified by Gram staining performed according to the conventional method and also with biochemical test (oxidase reaction). Both Gram-negative and oxidase-negative isolates were further tested. Biochemical tests other than oxidase test were done by using API 20E test kit (bioMérieux, Inc., France).

Samples were inoculated onto MacConkey agar (Oxoid), Xylosine lysine doxycyclate agar (Oxoid, UK), and Salmonella-Shigella agar (Oxoid, UK). *Shigella* like colonies were harvested with a sterile inoculating loop and transferred to 5% sheep blood agar and incubated 18-24 hours (overnight) at 37°C. Other plates of MacConkey's agar were inoculated to identify the Gram-negative organisms present in the samples. Analytical Profile Index (API) 20E biochemical panel was used for identification and differentiation of members of the family Enterobacteriaceae [12].

Moreover, bacterium that was confirmed as *E. coli* was subcultured onto Sorbitol MacConkey agar from nutrient agar and colorless colonies (nonsorbitol fermenter) were serologically confirmed using IGM antibodies to *E. coli* O157: H7 (*E. coli* O157: H7 test kit, Oxid). Serologic identification of *Shigella* is also performed by slide agglutination with

polyvalent somatic (O) antigen grouping sera.

2.7. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility tests were performed on Muller Hinton Agar (Oxoid, Hampshire, UK) by disc diffusion method. The following antimicrobial agents all from Oxoid were used: Ampicillin (10 µg), Amoxicillin(10µg), Trimethoprim/sulphamethoxazole (co-trimoxazole, 1.25/23.75 µg), Kanamycin(25µg), Chloramphenicol (30 µg), Ciprofloxacin (5 µg), Ceftriaxone (30 µg), Nalidixic acid (30 µg), Gentamicin (10 µg) and Tetracycline (30 µg). The resistance and sensitivity were interpreted according to the clinical and laboratory standards institute [13]. Antibiotics were selected based on local availability, effectiveness, literatures and, CLSI guidelines.

2.8. Quality Control

Stool specimens were collected by skilled laboratory technologist. Bacterial isolation and API E120 bio-chemical identification was examined by senior microbiologists and cross checked by microbiologist from the regional reference laboratory. All culture media were prepared following the manufacturers instruction and culturing procedures were done aseptically. Each batch of the prepared media was checked for sterility by incubating 5% of the batch at 37°C for one day. *E. coli* ATCC25922 was used as a control strains.

2.9. Data Analysis Procedures

Summary statistics such as frequencies and percentages were computed. Bivariate analysis was conducted primarily to check association of each variable with enteropathogenic bacteria growth. To control for possible effect of confounding, variables found to have association with the dependent variable at P-value of 0.25 in the bi-variate analysis was entered in to multiple logistic regression model. The variables showed significant association with p-value less than 0.05 in the multivariate logistic regression was considered to be independent factors.

3. Results

3.1. Socio Demographic Characteristics and Personal Hygiene Practice

A total of 398 food handlers participated in the study, giving the response rate of 91.3 %. The median age of the food handlers and their mean work experience was 22 years (\pm SD4.9) and 3 years (\pm SD2.1) respectively. Nearly a third (236; 59.3%) respondents were female and a significant proportion (56.3%) were completed secondary education. Around the quarter of the respondents 283(71.1%) were serving prepared food and 241(60.6%) were Wolaita in ethnicity.

The vast majority of the participants (369; 92.7%) were having a habit of eating raw meat and 223(56%) washed their hands after toilet with water. Of the total respondents, 225(56.5%) did not wash their hands after touching dirty

materials and 339(85.2%) did not use aprons. Moreover, 303(76.1%) had untrimmed finger nails, 318(79.95) did not get food hygiene training and 288(72.4%) did not have a medical checkup during the study period (Table: 1).

3.2. Enteric Bacterial Pathogens Prevalence

A total number of 35 entero-pathogens, 11 *Shigella spp.* and 24 *E. coli* O157: H7 were identified. Of the *Shigella spp.*, 6(11.11% of total isolates) were *S. flexneri*, the predominant isolates followed by *S. sonnei* 3(5.56% of total isolates). In this study 24(44.4% out of total isolates) and 92(26.1% out of the *E. coli* isolates were *E. coli* O157: H7.

Table 1. Socio demographic characteristics and Personal hygiene practice of food handlers (n=398) Wolaita Sodo town, Southern Ethiopia, 2015.

Socio-demographic characteristics	Frequency	Percent
Age		
< 20	92	23.1
21-35	301	75.6
>35	5	1.3
Mean	22	
Years of work (mean + SD)	3 \pm 2.1	
\leq 2years	135	33.9
>2years Median	263	76.1%
Sex		
Male	162	40.7
Female	236	59.3
Education		
No formal education	3	0.7
Primary education	52	13.1
Secondary education	224	56.3
Certificate and above	119	29.9
Occupation		
Cookers	65	16.3
Servers	283	71.1
Cleaners	50	12.6
Ethnicity		
Wolaita	241	60.6
Gammo	19	4.8
Gurage	77	19.3
Amhara	40	10.1
Other*	21	5.3
Habit of eating raw meat		
Yes	369	92.7
No	29	7.3
Hand wash after toilet		
No	93	23.4
With water	223	56
With Soap and Water	82	20.6
Hand wash after touching dirty materials		
No	225	56.5
With water	141	35.4
With Soap and Water	32	8
Use of apron/hair tie		
Observed	59	14.8
Not observed	339	85.2
Trimmed finger nails		
Yes	95	23.9
No	303	76.1
Food hygiene training		
Trained	80	20.1
Not trained	318	79.9
Medical check up		
Checked	110	27.6
Not checked	288	72.4

3.3. Factors Associated with Enteric Bacterial Pathogens Prevalence

Among all the factors associated with overall bacterial

prevalence, after excluding possible confounders, raw meat eating habit, hand washing after toilet and after touching dirty materials have shown significant association ($p < 0.05$) (Table 2).

Table 2. Factors associated with enteropathogenic bacteria isolated from 398 food handlers in Wolaita Sodo meal serving facilities from July 2014 to June 2015.

Associated factors	Bacterial growth	AOR (95 % CI)	P-value
Age			
≤20	92(23.1%)		*
>20	306(76.9%)		
Work experience			
≤2years	135(33.9%)		*
>2years	263(76.1%)		
Raw meat eating habit			
Yes	369(92.8%)	3.3(2.4-4.6)	0.001
No	29(7.2%)		
Hand wash after toilet			
No	93(23.4%)	2.31(1.34-3.84)	0.001
Yes	305(76.6%)		
Hand wash after touching dirty materials			
Yes	225(56.5%)	2.4(1.7-3.5)	0.001
No	173(43.5%)		
Trimmed finger nail			
Yes	95(23.9%)		
No	303(72.1%)		*
Food hygiene training			
Yes	80(20.1%)		
No	318(79.9%)		*

* No association statistically

3.4. Resistance Pattern to Antimicrobial Agents

Shigella isolates depicted high percentage of resistance to Ampicillin (90.9%), Amoxicillin (72.7%), Trimethoprim-sulfamethoxazole and Chloramphenicol each 63.6%. *Salmonella* isolate were also became highly resistant for Ampicillin (84.2%) and Amoxicillin (78.9%). High resistance level Ampicillin (87.5%) and Amoxicillin (75%) and low Ceftriaxone (12.5%) resistance *E. coli* O157: H7 isolates were also observed (Table: 3).

Table 3. Antimicrobial resistance pattern of enteropathogenic bacteria isolated from 398 food handlers in Wolaita Sodo food handling establishments from July 2014 to June 2015 Key.

Bacterial isolates	Resistance Number (%)									
	AMP	AMX	TMP-SXT	C	K	CIP	CRO	NA	CN	TTC
<i>Shigella</i> spp. (n=11)	10(90.9)	8(72.7)	7(63.6)	7(63.6)	4(36.4)	2(18.2)	2(18.2)	2(18.2)	3(27.3)	9(81.8)
<i>S. dysenteriae</i> (A)(n=1)	1(100)	1(100)	1(100)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)
<i>S. flexneri</i> (B)(n=6)	6(100)	4(66.7)	3(50)	3(50)	3(50)	2(33.3)	1(16.7)	1(16.7)	1(16.7)	5(83.3)
<i>S. boydii</i> (C)(n=1)	0(0)	1(100)	1(100)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	1(100)
<i>S. sonnei</i> (D)(n=3)	3(100)	2(66.7)	2(66.7)	3(100)	1(33.3)	0(0)	0(0)	1(33.3)	2(66.7)	2(66.7)
<i>E. coli</i> O157: H7(n=24)	21(87.5)	18(75)	10(41.7)	11(45.8)	5(20.8)	6(25)	3(12.5)	6(25)	5(20.8)	14(58.3)

3.5. Antibiogram Pattern of Multi-drug Resistant Bacteria

A total of 8(72.7%), and 14(58.3%) multidrug resistance *Shigella*, and *E. coli* O157: H7 were isolated respectively. Of *Shigella* species three isolates were resistant to five antibiotics and a single isolate for each was resistant to six, eight, nine and ten antibiotics. Of *E. coli* O157: H7 strain, 21(87.5%) isolates were resistance to one or more antibiotics, of which three isolates were resistance to nine antibiotics and two isolates were pan-resistant (Table: 4)

Table 4. Antibiogram pattern of enteropathogens isolated from 398 food handlers in Wolaita Sodo meal-serving establishments from July 2014 to June 2015.

Bacterial isolates	Pattern	Antibiotics	No (%)
<i>Shigella</i> spp. (n=11)	0	None	1(9.1)
	1	AMP	1(9.1)
	2	AMP, TTC	1(9.1)
	3	AMP, AMX, TTC	1(9.1)

Bacterial isolates	Pattern	Antibiotics	No (%)
<i>E. coli</i> O157: H7(n=24)	5	AMP,TTC,AMX,TMP-SXT,C	3(27.3)
	6	AMP,TTC,AMX,TMP-SXT,C,K	1(9.1)
	8	AMP,TTC,AMX,TMP-SXT,C,CIP, CN, K	1(9.1)
	9	AMP,TTC,AMX,TMP-SXT,C,K,CN,NA	1(9.1)
	10	AMP,TTC,AMX,TMP-SXT,C,K,CN,CRO,NA,CIP	1(9.1)
	0	None	3(12.5)
	1	AMP	3(12.5)
	2	AMP,AMX	4(16.7)
	3	AMP,AMX,TTC	2(8.3)
	4	AMP,AMX,TTC,C	1(4.2)
	5	AMP,AMX,TTC,TMP-SXT	1(4.2)
	5	AMP,AMX,TTC,C,NA	1(4.2)
	6	AMP,AMX,TTC,C,TMP-SXT	2(8.3)
	6	AMP,AMX,TTC,C,CIP,TMP-SXT	1(4.2)
9	AMP,AMX,TTC,C,TMP-SXT,CRO	1(4.2)	
9	AMP,AMX,TTC,C,TMP-SXT,CIP,NA,K,CN	3(12.5)	
10	AMP,AMX,TTC,C,TMP-SXT,CIP,NA,K,CN,CRO	2(8.3)	

4. Discussion

Overall prevalence of *Shigella* in the current study is comparable with two previous studies conducted in Gondar with the prevalence of 2.7% [8] and 3.1% [9] On the contrary, no *Shigella* species were revealed in previous studies conducted in Ethiopia [14] and Jordan [15]. Lower *Shigella* prevalence in Jordan as compared with our study could possibly due to the requirement for a health certificate and an annual examination of food handlers in hotels and restaurants in a previous study.

S. flexneri was the predominant *Shigella spp.* in this study which is corroborated with previous reports in North West [16], South West [17] and central Ethiopia [18] and Ghana [19] even though they noticed a much higher prevalence in line with our study. In the contrary to this study, *S. sonnei* was the predominant *spp.* according to a study conducted in South Ethiopia [20]. The wide variation of *Shigella* serogroups could be associated with difference in methodology, geographical difference and difference in strains circulating in local setups.

We also found in this study 6.03% *E. coli* O157: H7 which is higher than a study conducted in Jimma, Southwest Ethiopia with the prevalence of 1.8% [21]. This higher prevalence may be attributed to the very common culture of eating raw meat in the study area and some of food handlers selected in this study were working in restaurants selling raw meat for consumers. We recommend further study is mandatory for investigation to presence of *E. coli* O157:H7 in raw meats reaching to consumers.

There is high resistance of *Shigella* to commonly used antibiotic agents Ampicillin, Amoxicillin, Trimethoprim-Sulphamethoxazole, and Chloramphenicol, a finding corroborated with previous reports in Ethiopia [8], [9], [21], [22]. This finding is similar with studies done in different parts of Ethiopia.

Regarding resistance pattern of *E. coli* O157:H7 showed high resistance for Ampicillin (87.5%), Amoxicillin (75%),

and Tetracycline (58.3%), which is comparable with a study conducted in North East Ethiopia which shown high levels of antimicrobial resistance to Ampicillin (86.8%), Tetracycline (76%) and Trimethoprim-Sulfamethoxazole (76%) [23].

72.7% and 58.3% of the *Shigella spp.* and *E. coli* 157:H7 species were multidrug resistant and most of the strains were resistant to the most effective antibiotics available.

5. Conclusions

High resistant to Ampicillin and amoxicillin were observed in all species. Trimethoprim-Sulfamethoxazole and ciprofloxacin was the drug of choices for treatment of enteropathogenic bacteria. Raw meat eating habit and hand washing after toilet and after touching of dirty materials became predictor for enteric bacterial pathogens prevalence. Thus screening of food handles is important in order to prevent the transmission of enteric bacterial pathogens and treatment needs to be based on accurate laboratory detection of etiologic agents to mitigate the spread of drug resistant strains.

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