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Unhygienic Food Handling as a Source of Parasites and Pathogenic Bacteria in Dessie Town, North Eastern Ethiopia

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Abstract: Background: Food handlers (individuals engaged in food preparation, transport and provision) are implicated in the transmission of foodborne diseases if appropriate hygienic practices are not maintained. Hence, this study aimed to determine the prevalence of intestinal parasites and bacteria among food handlers of Dessie town, Ethiopia. Methods: A community based cross-sectional study was conducted among asymptomatic food handlers. Stool microscopy and culture was performed on 135 food handlers to determine intestinal parasites and enteric bacterial pathogens. Antimicrobial susceptibility pattern of the isolated bacteria was performed using the Kirby-Bauer disc diffusion method. Results: The majority of the food handlers were females (n=112; 83%), 18-27 years old (n= 95; 70.4%), with grade 9-12 education (n=56; 41.5%) and single in marital status (n= 87; 64.4%). The prevalence of intestinal parasites and enteric bacterial pathogens were 10.4% and 13.3%, respectively. *Entaemoeba histolytica/E. dispar,* (n=8; 5.9%) and *Shigella* species (n= 7; 5.2%) were the predominant parasitic and bacterial isolates respectively. Six (85.7%) of the *Shigella* isolates showed resistance to chloramphenicol and tetracycline while the single isolated *Pseudomonas* species showed resistance to all tested antimicrobials. Conclusion: Potentially contagious enteric bacterial pathogens and intestinal parasites were identified from food handlers who were presumed healthy. Hence, periodic screening of food handlers, and training on food handling and hand hygiene practices for food handlers is highly needed.

Keywords: Intestinal Parasite, Food Handler, Dessie Town, Antimicrobial Susceptibility, Enteric Bacterial Pathogens, Food Safety

1. Introduction

Safe food preparation requires good food handling practices, properly designed and constructed food preparation facilities and properly trained food handler [1]. Infectious diseases spread through food or beverages are a growing public health problems affecting both developed and developing countries [2, 3]. Foodborne or waterborne microbial diseases are responsible for nearly 1.9 million annual deaths worldwide [4]. In low-income settings, up to 70% of diarrheal diseases are associated with unsafe food consumption [5]. In this regard, concerns arise on the safety and sanitary conditions of food establishments and corresponding food handlers [6].

Bacteria, viruses and parasites are implicated in foodborne diseases, and staphylococcal food poisoning, salmonellosis, diarrhea associated with *Escherichia coli* (*E. coli)* and shigellosis are the leading reported causes [7]. Parasites important for food-borne transmission include *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium* species and helminthes including tapeworms, roundworms, and *Enterobius vermicularis* [8]. Morbidity and mortality associated with gastrointestinal infections may be increasing due to growing resistance of the etiologic agents for available antimicrobials [9, 10].

Contamination of food may occur at any point during its

journey through production, processing, distribution and preparation [11]. Food borne disease outbreaks originate either from food establishments serving unsafe food items for customers [12] or from direct contamination by food handlers [2]. Indeed, food handlers are implicated in the transmission of foodborne diseases to the public if appropriate hygienic practices are not maintained [13].

Therefore, knowledge of the epidemiology of etiologic agents and proper screening procedures is very important in order to detect infections among food handlers, thus preventing possible morbidity and protecting the health of consumers. Hence, this study was conducted to assess the profile of enteropathogenic bacteria and intestinal parasites among food handlers in Dessie town, Ethiopia.

2. Methods

2.1. Study Area and Period

This study was conducted from December 2013 - June 2014 in Dessie town, Ethiopia. Dessie town is found in Amhara regional state, 401km north of the capital city Addis Ababa.

2.2. Study Design

A community based cross-sectional study was conducted to assess intestinal parasites and enteric bacterial profile with their antimicrobial susceptibility patterns among asymptomatic food handlers of Dessie town.

2.3. Source Population

All food handlers in Dessie town were taken as source population for this study. Food handlers from the sampled food establishments who were willing to participate in this study were taken as study subjects. A preliminary survey was conducted in ten urban *kebeles (districts)* of Dessie town to identify the total number of food establishments and we found that, there was 349 food establishments (Hotel, Restaurant, Bar and Restaurant, Cafeteria, butcher house).

2.4. Sample Size Determination

The total sample size was obtained with the assumptions of power 80%, a 95% confidence interval and a marginal error of 5%. Then, a 10% non-response rate was added in order to get the total sample size of 135 food handlers.

2.5. Sampling Procedure

In order to come up with the total number of study subjects, we performed a preliminary survey in ten urban kebeles (districts) of Dessie town. We then performed proportional allocation to select a study participant through systematic sampling techniques. We randomly selected one food handler when there were more than one food handlers. Food handlers who were not available during the first and the second visit of data collection were excluded from the study.

2.6. Data and Sample Collections

Demographic characteristics of study subjects were collected using a structured questionnaire. A freshly passed stool sample was collected from all food handlers participating in this study.

The stool samples were transported immediately to Dessie Regional Health Research Laboratory and all the samples were processed and examined within 2 hours of collection to limit contamination and bacterial overgrowth.

2.7. Stool Processing for the Identification of Bacteria and Parasites

2.7.1. Culture and Identification

All stool specimens were inoculated into MacConkey's agar. Samples were also inoculated into Selenite F broth for enrichment and were sub-cultured onto xylose lysine deoxycholate (XLD) agar after 6 hours of incubation at 37°C (WHO CDD/83.3). The primary plates and the subculture plates from the enrichment media were incubated for 24 hours at 37°C. After 24 hours, isolates were identified following standard procedures using colony characteristics and biochemical tests. The series of biochemical tests used in the identification of the isolates included: hydrogen sulfide production, indole production, motility in Sulfide-Indole-Motility (SIM) medium, citrate utilization, urease production, different carbohydrate fermentation reactions and lysine decarboxylase (LDC) in Simmon's citrate agar, Urea agar, Kligler's iron agar (KIA) and lysine iron agar (LIA) (WHO, 2003).

2.7.2. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed for the bacterial isolates according to the criteria of the Clinical and Laboratory Standards Institute (CLSI, 2011) by the Kirby-Bauer disc diffusion method. The following antimicrobial agents (Oxoid, UK) were used to test the isolates: amoxicillin-clavulanic acid (30μg), ampicillin (10μg), chloramphenicol (30μg), ciprofloxacin (5μg), gentamicin (10μg), ceftriaxone (30μg), nalidixic acid (30μg), trimethoprim-sulfamethoxazole (25μg) and tetracycline (30μg). The resistance and sensitivity were interpreted according to the Standards. The zone of inhibition diameters around the antibiotic discs were measured using a ruler and reported as sensitive, intermediate, and resistant according to the Standards.

2.7.3. Microscopic Examination of Stool

Microscopic examination of stool was performed in Dessie Regional Health Research Laboratory using smears from direct wet mount preparations (both saline and iodine) and formalin-ether concentration techniques. Standard operational- procedures were followed to ensure the detection of trophozoites, larvae, cysts and ova [16]. Both saline and iodine wet mounts were prepared on a single microscopic slide using the left half side for saline (0.85% NaCl) and the right half side for Lugol's iodine. Slides were examined under 10× and 40× objective lenses of a light

microscope by two experienced microscopists. Two wet mount slides (one for saline/iodine and the other for formalin-ether concentration) were prepared and each slide was examined by two microscopists. Finally, results agreed by both microscopists were documented.

2.8. Statistical Analysis

Demographic data and findings from stool microscopy and stool culture were entered using SPSS version 20.0 and then cleaned before analysis. Means (continuous variables) and proportions (categorical variables) were used as descriptive measures. Variables with p-value<0.2 in bivariate analysis were included in multivariable logistic regressions. Variables with p-value< 0.05 in the final model were considered to be statistically significant and independently associated with occurrence of intestinal parasites and enteric bacterial pathogens.

3. Results

3.1. Socio-demographic Data

A total of 135 food handlers were enrolled in this study. The majority of study participants were female (n=112; 83%), 18-27 years old (n=95; 70.4%), single in marital status (n=87; 64.4%) and with grade 9-12 education (n=56; 41.5%) (Table 1). The mean age and mean monthly income of food handlers in this study were 25 ± 9 years and 27.5±23.2 US$, respectively.

Table 1. *Enteric bacterial pathogens and intestinal parasites with demographic characteristics of food handlers in Dessie town, Ethiopia (n=135).*

Variables	Total	Enteric bacterial pathogens		Intestinal parasites	
		Yes	No	Yes	No
Sex					
Female	112(83%)	15(13.4%)	97(86.6%)	13(11.6%)	99(88.4%)
Male	23(17%)	3(13.0%)	20(87.0%)	1(4.3%)	22(95.7%)
Age group					
14-17	10(7.4%)	1(10.0%)	9(90.0%)	1(10.0%)	9(90.0%)
18 - 27	95(70.4%)	12(12.6%)	83(87.4%)	11(11.6%)	84(88.4%)
28-37	18(13.3%)	1(5.6%)	17(94.4%)	1(5.6%)	17(94.4%)
38-47	6(4.4%)	2(33.3%)	4(66.7%)	0(0%)	6(100%)
48+	6(4.4%)	2(33.3%)	4(66.7%)	1(16.7%)	5(83.3%)
Religion					
Orthodox	84(62.2%)	15(17.9%)	69(82.1%)	10(11.9%)	74(88.1%)
Muslim	45(33.3%)	2(4.4%)	43(95.6%)	4(8.9%)	41(91.1%)
Protestant	4(3%)	0(0%)	4(100%)	0(0%)	4(100.0%)
Others	2(1.5%)	1(50.0%)	1(50.0%)	0(0%)	2(100.0%)
Educational status					
Illiterate	17(12.6%)	3(17.6%)	14(82.4%)	5(29.4%)	12(70.6%)
Grade 1-4	7(5.2%)	2(28.6%)	5(71.4%)	0(0%)	7(100.0%)
Grade 5-8	42(31%)	5(11.9%)	37(88.1%)	5(11.9%)	37(88.1%)
Grade 9-12	56(41.5%)	6(10.7%)	50(89.3%)	2(3.6%)	54(96.4%)
College/above	13(9.6%)	2(15.4%)	11(84.6%)	2(15.4%)	11(84.6%)
Marital status					
Single	87(64.4%)	9(10.3%)	78(89.7%)	8(9.2%)	79(90.8%)
Married	33(24.4%)	7(21.2%)	26(78.8%)	3(9.1%)	30(90.9%)
Divorced	14(10.4%)	2(14.3%)	12(85.7%)	3(21.4%)	11(78.6%)
Widowed	1(0.7%)	0(0%)	1(100.0%)	0(0%)	1(100%)
Monthly income					
≤21 USD	79(58.5%)	10(12.7%)	69(87.3%)	10(12.7%)	69(87.3%)
>21 USD	56(41.5%)	8(14.3%)	48(85.7%)	4(7.1%)	52(92.9%)

3.2. Stool Microscopy

Microscopic examination of stool identified 14(10.4%) food handlers with five different species of intestinal parasites.

Table 2. *Intestinal parasites identified from food handlers of Dessie town, Ethiopia.*

Intestinal Parasites	Frequency (n=135)	Percent (%)
Entamoeba histolytica/ E. dispar	8	5.9
Giardia lamblia	3	2.2
Taenia species	1	0.7
Strongyloides stercoralis	1	0.7
Trichuris trichiura	1	0.7

As shown in Table 2, *E. histolytica/E. dispar* were the leading intestinal parasites 8(5.9%) followed by *G. lamblia* 3(2.2%).

3.3. Enteric Bacterial Pathogens and Their Antimicrobial Susceptibility Patterns

A total of 18(13.3%) enteric bacterial pathogens within six different bacterial species were isolated from stool cultures. *Shigella* species were the predominant isolate 7(5.2%) followed by *Proteus* species 4(3%) and *Citrobacter* species 3(2.2%) (Table 3).

All (100%) of the *Shigella* isolates were sensitive to two antimicrobials: ciprofloxacin and trimethoprim-sulfamethoxazole. However, 6(85.7%) of the *Shigella* isolates showed resistance to chloramphenicol and

tetracycline. The single *Pseudomonas* isolate (100%) showed resistance to all tested antimicrobials. The single *Enterobacter* isolate showed sensitivity to all of the tested antimicrobials. Two (100%) of the *Klebsiella* species isolates were resistant for ampicillin but one isolate (50%) was resistant for both chloramphenicol and gentamycin.

Table 3. *Enteric bacterial pathogens and their antimicrobial susceptibility patterns among food handlers of Dessie town, Ethiopia (n=18).*

Enteric bacterial pathogens	n (%)	Sensitivity pattern	Antimicrobial agents								
			AML	AMP	CRO	C	CIP	TS	CN	NA	TET
			n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Shigella spp.	7(5.2)	Susceptible	3(43)	2(29)	7(100)	1(14.3)	7(100)	7(100)	6(85.7)	7(100)	1(14.3)
		Resistant	4(57)	5(71)	0	6(85.7)	0	0	1(14.3)	0	6(85.7)
Proteus spp.	4(3.0)	S	4(100)	3(75)	4(100)	1(25)	4(100)	4(100)	4(100)	(100)	4(100)
		R	0	1(25)	0	3(75)	0	0	0	0	0
Citrobacter spp.	3(2.2)	S	1(33.3)	2(66.7)	7(100)	1(33.3)	3(100)	3(100)	3(100)	3(100)	1(33.3)
		R	2(66.7)	1(33.3)	0	2(66.7)	0	0	0	0	2(66.7)
Klebsiella spp.	2(1.5)	S	1(50)	0	0	1(50)	2(100)	2(100)	1(50)	2(100)	1(50)
		R	1(50)	2(100)	2(100)	1(50)	0	0	1(50)	0	1(50)
Pseudomonas spp.	1(0.7)	S	0	0	0	0	0	0	0	0	0
		R	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
Enterobacter spp.	1(0.7)	S	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
		R	0	0	0	0	0	0	0	0	0

AML = Amoxycillin-Clavulanic acid, AMP = Ampicillin, CRO = Ceftriaxone, C = Chloramphenicol, CIP = Ciprofloxacin, TS = Trimethoprim-Sulfamethoxazole, CN = Gentamicin, NA = Nalidixic Acid, TET = Tetracycline, R = Resistant, S = Sensitive

The mean age of food handlers with enteric bacterial pathogens (28.4 ± 12 years) were significantly higher than those without enteric bacterial pathogens (24.2 ± 8 years). Moreover, there was significant difference in mean monthly income of food handlers with and without intestinal parasites (Figure 1).

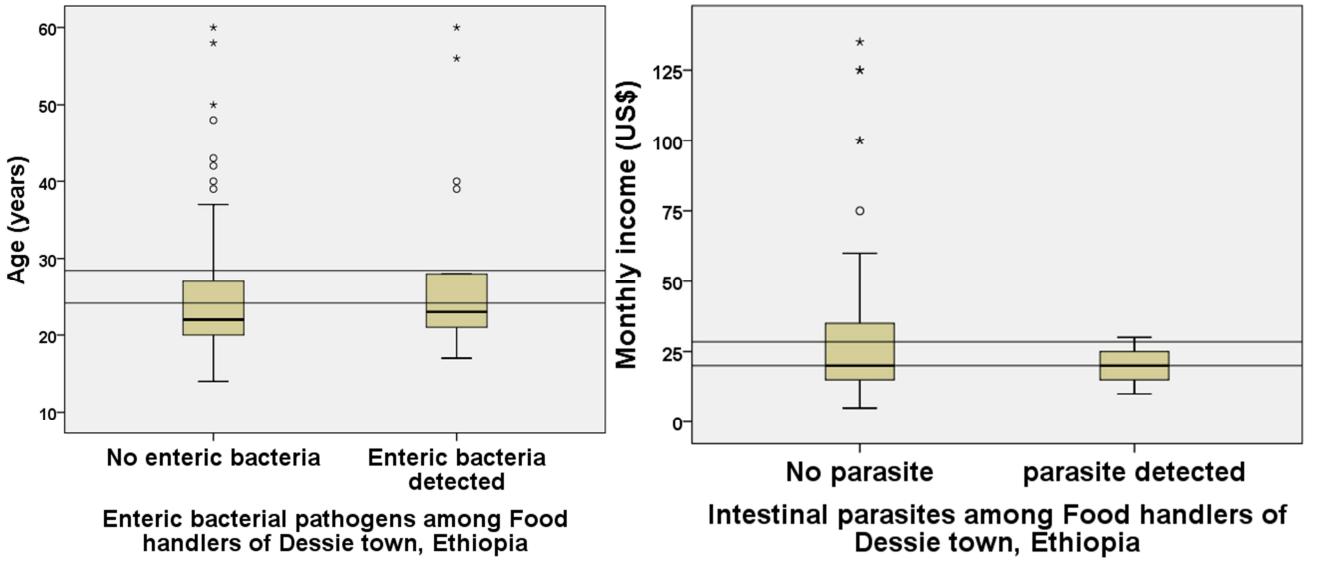

Figure 1. *(A) The mean age difference of Food handlers with and without enteric bacterial pathogens (p=0.05), (B) The mean difference in monthly income of Food handlers with and without intestinal parasites (p=0.003).*

4. Discussion

Intestinal parasitic prevalence among food handlers in this study was 10.4%. This finding was much lower than a finding in Jimma [17] and Bahir Dar [18] in which intestinal parasitic prevalence among food handlers were 45.5% and 41.1%, respectively. This might be due to high intestinal parasitic prevalence in the general population of Jimma and Bahir Dar town [19, 20].

Moreover, the high altitude of Dessie town (2,470 m) may not be favorable for parasites as compared to lower altitude in Jimma town (1,780 m) and Bahir Dar town (1,800 m).

E. histolytica/E. dispar was the predominant intestinal parasite among food handlers in this study. This finding is in agreement with findings in Ethiopia and Kenya [17, 21- 22] in which majority of identified intestinal parasites among food handlers were *E. histolytica/E. dispar*. This might be explained by the fact that *E. histolytica/E. dispar* is dominantly found in the environment as compared to other intestinal parasites.

In the present study, the prevalence of enteric bacterial pathogens was 13.3%. This finding is higher than findings in Jimma [17, 23] in which enteric bacterial pathogens among food handlers were reported to be 3.5% and 6.9%, respectively. The reason for the difference in the prevalence

of enteric bacterial pathogens in the stools might be due to variation in climate, geography and study settings.

From our study, isolation rate of *Shigella* species from the asymptomatic food handlers was found to be 5.2%, and this is higher than the results from Jimma (0.9%), Western Ethiopia and Arba Minch (3%), Southern Ethiopia [21, 24]. This may be due to difference in sample size and technique. As *Shigella* organisms do not have any natural reservoirs in animals, they spread only from person to person. So it is very important to note the hygienic practice of food handlers.

All *Shigella* isolates in our study were sensitive to ciprofloxacin and trimethoprim-Sulfamethoxazole and shown higher resistance to chloramphenicol and tetracycline. Our finding is comparable to the study conducted in Gondar University Cafeteria, Northwest Ethiopia [25] which shows high resistance to tetracycline (90%), chloramphenicol (67.8%) and 100% sensitivity to ciprofloxacin.

The rates of isolation of other bacteria from stool cultures (Table 3) are nearly in line with the study conducted in Gondar University, [25] reported as *Klebsiella* species (1.6%), *Pseudomonas* species (0.67) *Citrobacter* species (0.8%), and *Enterobacter* species (0.8%). The detection of these enteropathogenic bacteria from food handlers may pose significant risk for the consumers of the food establishments because the organisms cause food poisoning.

5. Conclusion

It is evident that potentially contagious enteric bacterial pathogens and intestinal parasites were identified from food handlers which were presumed healthy. Hence, periodic screening of food handlers is highly needed. In this regard, the local government authorities alongside the owners of food establishments should strengthen awareness of the importance of periodic medical screening of food handlers.

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Competing Interests

Authors declare that there is no conflict of interest

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