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Bacterial Profile and Antimicrobial Susceptibility Pattern among Food Handlers at Gondar University Cafeteria, Northwest Ethiopia

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Abstract

Background: Salmonellosis and shigellosis remain a major public health problem across the globe. The problem is severe in developing countries due to difficulties in securing optimal hygienic food handling practices. The aim of this study was to assess the prevalence of *Salmonella*, *Shigella* other public health important bacteria and their susceptibility pattern, and hygienic practice of the Food handlers at the University of Gondar cafeteria.

Methods: A cross sectional study was conducted. Serum, stool and finger nail swabs were investigated in respective of the procedure for *Salmonella, Shigella* and other public health important bacteria following standard laboratory procedures.

Results: Of 300 food handlers, 256 (85.3%) were females. Only 27.5% of the food handlers knew that infected food handlers are risk for food contamination. Eight (2.7%) *Shigella* species and 4 (1.3%) *Salmonella Typhi* were isolated from stool cultures of food handlers. Coagulase-negative staphylococci were the predominant bacteria species (33%) isolated from finger nail bed, followed by *S. aureus* (16%), *E. coli* (2.67%). and *Klebsiella* species (1.67%). All isolated *Salmonella Typhi* were resistant to chloramphenicol (100%). All *Shigella* species were sensitive to ciprofloxacin. Three (6.3%) of *S.aureus* isolates were resistant for methicilin.

Conclusion: Isolation of *Salmonella*, *Shigella* and other public health important bacteria from food handlers may pose significant risk on the consumers. Therefore, it is essential for University of Gondar to implement food handlers training on food safety, conduct periodic medical checkup and continuous monitoring of personal hygiene.

Keywords: Food handlers; *Salmonella*; *Shigella*

Introduction

Food related infections constitute an important public health problem in both developed and developing countries [1]. The problem is severe in developing countries due to difficulties in securing optimal hygienic food handling practices. In developing countries, up to an estimated 70% of cases of diarrheal disease are associated with the consumption of contaminated food [2].

Salmonella Typhi is one of the major causes of food and water borne gastroenteritis in human and remains an important health problem worldwide [3]. The World Health Organization estimates 16 million new cases and 600,000 deaths of typhoid fever were each year worldwide [4]. The emergence of drug resistance to *S. Typhi* has been of major concern in recent years [5]. *S. Typhi* has been showed high resistances against ampicillin, cotrimoxazole, tetracycline, and chloramphenicol, genentamicin and norfloxacillin [6].

Several studies have demonstrated that food handlers harbour *S. Typhi* asymptomatically [7]. One of the historically notorious examples is that of the American cook "Typhoid Mary" (Mary Malon) who was responsible for 7 epidemics of typhoid fever affecting more than 200 persons [8].

Accordingly, food handlers with poor personal hygiene and inadequate knowledge working in food serving establishments could be potential sources of infections of many enteropathogenic bacteria and parasites [9,10]. Likewise, food handlers who harbour enteropathogenic bacteria may contaminate foods with their faeces via their fingers, which in turn lead to food processing, and finally to infection of consumers [11]. Compared to other parts of the hand, the area beneath fingernails harbours many microorganisms and is difficult to clean [12]. More aggravated situations and challenges prevail in Ethiopia where food safety issues are not well understood and have received little attention. The aim of this study is therefore, to assess the prevalence of *Salmonella, Shigella*, other public health important bacteria and their antimicrobial susceptibility pattern among food handlers, and hygienic practice of the food handlers and their association to pathogens among food handlers at the University of Gondar cafeteria.

Materials and Methods

Study design, area, period and population

A cross sectional study was conducted at University of Gondar, which is located in Amhara region (Northwest Ethiopia) from March 1 to July 30, 2012. All food handlers working at the four University cafeterias (College of Medicine and Health Sciences, Tewodros, Maraki and the Hospital cafeteria) were included in the study (n=300). At present, University of Gondar has about 22,000 students in regular and extended programs and cafeterias currently serve meals for (15,000 students and 500 patients).

Data and sample collections

A structured questionnaire was used for collecting information on demographic characteristics, knowledge, practice and hygienic status of each food-handler. Stool specimen was collected from each

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food handler with leak proof stool cup by experienced laboratory technologist. The collected samples was immediately inoculated on to selenite F broth (Oxoid UK) and transported to Microbiology laboratory for 5 minutes. Two milliliters of venous blood was collected by laboratory technologist from food handlers for Widal test. Finger nail swabs were collected from food handler of both hand finger nail contents by sterile applicator stick tipped with cotton and moistened with normal saline. These samples were kept with normal saline in a test tube until they were inoculated onto the respective culture media not more than 5 minutes.

Stool samples culture and identification of *Salmonella* and *Shigella* spp.

For isolation of *Salmonella* and *Shigella* species, stool samples were enriched in Selenite F broth for 18 hours prior to inoculating onto the plates of Salmonella-Shigella agar (Oxoid, UK). After 24 hours of incubation at 37°C, isolates were identified following the standard procedures using biochemical tests such as indole, motility, lysine decarboxylase, triple sugar iron agar, citrate and urea [13].

Processing of fingernail swabs and identification of bacteria and parasites

A single finger swab obtained from each food handler was cultured immediately on Manitol salt agar (MSA) and Blood agar plate (BAP) for isolation of *S. aureus* and Coagulase negative *Staphylococci* (CNS). Finger nail swabs were cultured onto Blood agar plate (BAP) and MacConkey agar and then incubated at 37°C for 24 hours for isolation of Gram negative bacteria such as *Pseudomonas aeroginosa*, *Escherichia coli*, and other bacteria. Isolates were identified by growth characteristics on respective culture media and by doing biochemical tests following standard procedures [13]. Intestinal parasites were investigated microscopically from each finger nail swab using direct wet mount following laboratory standards [14].

Antimicrobial susceptibility testing

Antimicrobial susceptibility tests were performed on Muller Hinton Agar (Oxoid, Hampshire, UK) by disc diffusion method. The following antimicrobial agents were used for Gram positive isolates: Methicilin (10 µg), penicillin (10 µg), erythromycin (15 µg), ampicilin (30 µg), ciprofloxacin (10 µg), tetracycline (30 µg), cotrimoxazole (25 µg), and vancomycin (30 µg).To characterize Gram negative isolates, we used ampicilin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), gentamycin (10 µg) and norfloxacillin (10 µg) cotrimoxazole (25 µg) and ciprofloxacin (10 µg). The resistance and sensitivity were interpreted according to the National Committee for Clinical Laboratory Standards [15].

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 a sample medium at 37°C for one day. *E. coli* ATCC25922 and *S. aureus* ATCC25923 sensitive to all antimicrobial agents were used as a control strains.

Serum processing and Widal test

Widal test was done using *S. Typhi* O and H antigens according to the manufacturer's instruction for the diagnosis of *S. Typhi*. In brief, the test was done by mixing one drop of serum with one drop each of O and H antigens separately on glass slide. After shaking the slide back and forth, the mixture was observed for macroscopic agglutination. If there was agglutination within one minute it was reported as positive, otherwise as negative.

Data processing and analysis

Statistical analysis was done using SPSS version 16.00 software. The chi-square test was employed to assess the association between variables. A p-value of less than 0.05 was considered to indicate statistical significance.

Ethical Considerations

The data were collected after written informed consent obtained from all study participants, and the study was approved by the Research Ethics Committee of the University of Gondar. Sample taken from each patient was coded and results obtained from each patient were kept always confidential. The food handlers who were positive for *Salmonella* and *Shigella* were treated at the University of Gondar Hospital.

Results

Sociodemographic characteristics

The study included all 300 food handlers, and of which, 256 (85.3%) were females. Their mean age were 31.07 years, ranging from 18-68 years. The majority 202 (67.3%) of the food handlers were young adults aged 18-39 years. Majority of the food handlers 219 (71.7%) had 1-10 years of work experience (Table 1).

Knowledge and practice of food handlers on food hygiene

Two hundred sixty five (88.3%) of the food handlers knew at least one type of food borne disease. Of those who were asked about mode of transmission of food borne diseases, 74.7% answered that contaminated food was the vehicle. Only 27.5% of the food handlers were knew that infected food handlers are risk for food contamination (Table 2).

In hand washing assessment, 272 (90.6%) study participants had a habit of hand washing after toilet. Two hundred forty seven (82.3%) of food handlers had the habit of hand washing with soap and water. However, 145(45%) of them had a habit of hand washing

Characteristics	No	%
Age in years		
18-28	127	42.3
29-39	75	25
40-49	47	15.7
50-59	47	15.7
60+	4	1.3
Sex		
Female	256	85.3
Male	44	14.7
Education		
Illiterate	17	5.7
Grade 1-8	126	42
Grade 9-12	129	43
Certificate	28	9.3
Service Years		
<1 years	36	12
1-10 years	219	71.7
11-20 years	18	6
Marital status		
Married	182	60.7
Single	87	29
Divorced	31	10.3

Table 1: Sociodemographic characteristics of food handlers working at cafeteria of Gondar University, February1 to June 30, 2012 (n=300).

after touching nose between handling of food items. Only 12(4%) of study participants were certified for training in food handling and preparation. One hundred twenty six (87%) of the food handlers had`t got service training.

Carriers of *Salmonella* and *Shigella*

Stool cultures revealed 2.7% of *Shigella* species. All *Shigella* species were resistant to ampicilin. However; all *Shigella* species were sensitive to ciprofloxacin (Table 3). Stool cultures of food handlers also revealed 4(1.3%) of *S. Typhi*. All isolated *S. Typhi* were resistant to chloramphenicol (100%). Two of the isolates were resistant to cotrimoxazole (50%). However, all *S. Typhi* were sensitive to ciprofloxacin and norfloxacin. The serum of the food handlers were screened by Widal test. The results showed that, 42 (14%), 30 (10%), and 18 (6%) of the food handlers were positive for O, H and for both antigens; respectively.

In this study, the study participant who have not certified in food preparation and handling more likely carriers for *Salmonella* and *Shigella* than that of the certified study participants (P=0.0005) (Table 4).

Finger nail bed contents of bacteria

Cultures of fingernail contents were found to be positive for different bacterial species. Coagulase-negative *Staphylococci* were the predominant bacterial species (33%) isolated followed by *S. aureus* (16%) (Table 5).

Most of the *S. aureus* isolates were resistant to ampicilin and penicillin (54.2%), followed by amoxicillin (45.8%). However, all isolates of *S. aureus* were sensitive to vancomycin. Of the total 99 isolates of coagulase negative *Staphylococcus* species, 45.5% were resistance to penicillin and ampicillin, followed by amoxicillin (39.4%) (Table 6). The antibiotic resistance pattern of Gram negative bacteria isolated from finger nail contents were shown in table 6.

Knowledge	Frequency	Percent (%)
Heard about food borne disease	265	88.3
Have no heard about food borne disease	35	11.7
Cause of food borne disease		
Germs	163	61.5
Chemicals	60	22.6
Unhygienic food preparation	185	69.8
Anger of God	15	5.6
Mode of food borne disease transmition		
Contaminated food	198	74.7
Contaminated water	154	58.1
Vectors like cockroaches/flies	55	20.7
Reasons for food contamination		
Contaminated hand	243	91.7
Infected food handler	73	27.5
Unclean utensil	163	61.5

NB: Because of the possibility of multiple responses total number of food handlers may not be equal to 300.

Table 2: Food handlers' knowledge about food borne disease, working at University of Gondar cafeteria, fromFebruary1 to June 30, 2012 (n=300).

No intestinal parasites were detected in finger nail content of food handlers in this study.

Discussion

In this study, stool culture, fingernail bed swab culture and microscopic examination food handlers were investigated for the presence of bacteria and intestinal parasites. The result showed that food handlers were positive for *Shigella* species, *S. Typhi*, and other public health important bacteria. These indicate that the hygienic practice of the food handlers working in catering establishments of the study site is not satisfactory.

Isolation rate of *Shigella* species in our study is comparable with a study done previously in Gondar (3.1%) from food handlers [9]; however, it is lower than the finding reported as 90 (7.5%) in pediatric patients in the University of Gondar Hospital [16]. This may be due to the difference in technique, type of study participant and the sample size. The present study demonstrated higher isolates of *Shigella* species compared to reports from Sudan (1.3%) and Jordan (1.4%) [17,18]. It is important to note that *Shigella* organisms do not have any natural reservoirs in animals and spread only from person to person and outbreak are most often in facilities such as day care center, and cafeterias, and similar settings [19].

All *Shigella* isolates in our study were sensitive to ciprofloxacin and shown lower resistance to norfloxacin. However, the isolates showed high resistance for commonly used antibiotic agents (Table 3). This finding is comparable to a study reported in the University of Gondar Hospital [16] which shows high resistance to tetracycline (90%), cotrimoxazole (84.6%), ampicilin (78.9%) and chloramphenicol (67.8%) and lower resistance to gentamycin (12.2%), ciprofloxacin (2.2%) and norfloxacin (1.1%).

Prevalence of *S. Typhi* in this study is comparable with a report from Japan (0.032%) and Bahir Dar 6(1.6%) [6,20]. In the meantime, this study showed that, 42(14%), 30(10%), and 18(6%) food handlers were positive for O, H and both, respectively. However, specificity of Widal test is low for the diagnosis of *Salmonella Typhi.*

To the contrary, *Salmonella* species was not isolated in Gondar town [9]. Although the source of *Salmonella* species is intestinal tract of animals and humans, their carriage in food handlers was very rare [19]. However, a sudden outbreak of food poisoning due to *Salmonella* Newport was reported as 79(23%) ten years ago, in the students population of Gondar College of Medical Sciences [21]. The food handlers, from whom *Salmonella* species was isolated, may be a potential risk group for a sudden outbreak of salmonella food poisoning in the students' population. *S. Typhi* showed high resistance for commonly used antimicrobial which is comparable to the study done in Bahir Dar [6] indicated that antimicrobial resistance of *S. Typhi* is an increasing concern.

The rates of isolation of bacteria from the finger nail content cultures (Table 3) are nearly in line with the study conducted in Gondar town previously [9] reported as coagulase negative staphylococci (41.7%), *S. aureus* (16.5%), *Klebsiella* species (5.5%), *E. coli* (3.1%), *Serratia* species (1.58%), *Citrobacter* species (0.8%), and *Enterobacter* species (0.8%). Especially, the detection of *S. aureus* in finger nail of food handlers may pose significant risk for the consumers of the cafeterias because the organism may produce enterotoxins and causes food poisoning.

This study showed that the presence of Gram negative bacterial species in the finger nails may explain the contamination of fecal matter. This specifies that the sanitation of the environment and food handlers are in question. The presence of *E. coli* in the food handler`s

Bacterial isolate	Total	Sensitivity Pattern.n(%) n	Antimicrobial agents tested						
			Ampicili	Gentam	Tetracy.	Ciprof.	Cotrimo-xazole	Chlora.	Norflo.
E.coli	8	S	3(37.5)	5(62.5)	4(50)	8(100)	6(75)	4(50)	8(100)
		R	5(62.5)	3(37.5)	4(50)	0	2(25)	4(50)	0
***Klebsiella* spp.**	5	S	2(40)	3(60)	4(80)	5(100)	3(60)	3(60)	5(100
		R	3(60)	2(40)	1(20)	0	2(40)	2(40)	0
***Citrobacter* spp.**	3	S	2(66.7)	3(100)	2(75)	3(100)	3(100)	1(33.3)	3 (100)
		R	1(33.3)	0	1(25)	0	0	2(66.7	0
***Enterobacter* spp**	2	S	2(100)	2(100)	2(100)	2(100)	2(100)	1(50)	2(100)
		R	0	0	0	0	0	1(50)	
P. aeroginosa	2	S	0	2(100)	0	0	0	0	0
		R	2(100)	0	2(100)	2(100)	2(100)	2(100)	2(100)
S. Typhi*	4	S	1(25)	3(75)	2(50)	4(100)	2(50)	0	4(100)
		R	3(75)	1(25)	2(50)	0	2(50)	4(100)	0
Shigella spp*	8	S	0	6(75)	2(25)	8(100)	4(50)	2(25)	7(87.5)
		R	8(100)	2(25)	6(75)	0	4(50	6(75)	1(12.5)

n =Number R= Resistance S= Sensitive *=found in stool culture only

Table 3: Antimicrobial resistance pattern of different Gram negative bacterial species isolated from finger nail content and stool cultures of food handlers working at the students' cafeterias and Hospital cafeteria of Gondar University, February1 to June 30, 2012 (n=300).

Variables	*Shigella* and *Salmonella*		OR	95%	
Certified in food preparation and handling	**Pos(%)**	**Neg (%)**			P value
Yes	3(25)	9(3.1)	10.3	(1.9, 53.1)	0.00015*
No	9(75)	279(96.9)			
Medical check up					
Yes	4(33.3)	176(61.1)	0.32	(0.08,1.2)	0.054*
No	8(66.7)	112(38.9)			
Hand washing after toilet by water					
Yes	9(75)	263(91.3)	0.29	(0.05,1.43)	0.056*
No	3(25)	25(8.7)			
Hand washing after using toilet with soap and water					
Yes	7(63.6)	240(83.3)	0 .28	(0.08,1.07)	0.026*
No	5(36.4)	48(16.7)			
Hand washing after touching nose					
Yes	6(50)	139(48.3)	1.07	(0.30,3.86)	0.91**
No	6(50)	149(51.7)			
Hand washing before preparing food					
Yes	10(83.3)	265(92.3)	0.45	(0.08,3.06)	0.26*
No	**2(16.7)**	**23(7.7)**			

OR= odds ratio
*=Fisher`s exact test
**=Chi-square

Table 4: Hygienic practice of food handlers, in relation to *Salmonella Typhi* and *Shigella* species, working at cafeteria of Gondar University from February 1 to June 30, 2012 (N=300).

finger nail bed contents may be involved for food contamination in the students population and patients. A study showed that *E. coli* O157: H7 was one of the most commonly recognized food borne infections [22]. However, in the present study, no *Shigella* and *Salmonella* were isolated from finger nails of food handlers. This is consistent with the previous study done in Gondar [9].

The *S. aureus* and coagulase negative staphylococcus found in the finger nail content were resistant to multiple antibiotics in this study. *S. aureus* isolated from finger nail contents were resistant to methicilin. If it is transmitted to patients, it may cause epidemics in patients.

Stages of intestinal parasites were not detected in the fingernails of

Type of organism	N0.	%
S. aureus	48	16
Coagulase-ve *staphylococci*	99	33
E.coli	8	2.67
***Citrobacter* spp.**	3	1
***Klebsiella* spp.**	5	1.67
Pseudomonas aeroginosa	2	0.67
***Entrobacter* spp.**	2	0.67
Total	**167**	**55.7**

Table 5: Types of bacteria isolated from finger nail cultures of food handlers working at the cafeteria of Gondar University, February 1 to June 30, 2012 (n=300).

Antimicrobial agents tested	Sensitivity pattern	*S. aureus* (n=48) N0.(%)	CNS (n=99) N0.(%)
Vancomycin	R	0	0
	S	100	100
Methicilin	R	3(6.3)	4(4.0)
	S	45(94.7)	95(96.0)
Ampicilin	R	26(54.2)	45(45.5)
	S	22(43.8.)	54(54.5)
Penicillin	R	26(54.2)	45(45.5)
	S	22(43.8)	54(54.5)
Amoxicillin	R	25(52.1)	39 (39.4)
	S	23(47.9)	60(60.6)
Tetracycline	R	11(21.9)	17(17.2)
	S	37(78.1)	82(82.8)
Ciprofloxacin	R	8(16.7)	7(10.6)
	S	40(83.3)	59(89.4)
Cotrimoxazole	R	11(21.9)	15(15.2)
	S	37(78.1)	84(84.8)
Erythromycin	R	9(18.8%)	14(13.6)
	S	39(81.2)	85(86.4)

CNS=Coagulase negative *Staphylococci,* N=Number, R=Resistant, S=Sensitive

Table 6: Antimicrobial resistance pattern of *S. aureus* and CNS isolated from finger nail cultures of food handlers working at cafeteria of Gondar University, February1 to June 30, 2012 (n=300).

food handlers in the present study. This finding is in line with the result obtained from study done earlier in Gondar town [9]. However, other earlier report in Jimma showed the presence of ova, larvae, and cysts of intestinal parasites under fingernails of study participants [23].

More than half of the food handlers had medical checkup in the past which is higher than a report from Mekelle town (22.7%) [24]. However, none of the food handlers had medical checkup in a study conducted in Bahir Dar [6].

Food handlers hand washing practices after toilet is high, which is in agreement with previously reported in Gondar town (89.5%) and (89%) [6,9], and in Bahir Dar [25]. However, nearly half of the food handlers in our study practice hand washing after touching nose and between handling of food items in this study. These reflected that the food handlers lack of awareness about food contamination with poor hygienic practices. Health education intervention on food safety and hygiene must be strengthened to ensure food safety during processing, preparation and storage in food service establishments. The study done in Nigeria showed that 71.9% of the food handlers washed their hands with soap and water which is slightly lower than our finding while 28.1% of Nigerians washed their hands with only water [26] that is higher than our report.

Conclusion

High number of *Shigella* species, *Salmonella Typhi* and other public health important bacteria were detected among food handlers. The bacterial isolates showed high antibiotic resistance pattern. This indicates that the food handlers may transmit food borne disease for the student populations and patients being served in the cafeterias. Food handlers who harbored *Salmonella* and *Shigella* should be excluded from work until they are treated and completely cured. Those food handlers should have medical checkup before employment. Treatment of food handlers who are cases of *Salmonella* and *Shigella* would be based on antibiotic susceptibility of the isolates so as to decrease resistance challenge. Further study is recommended to check the presence of microorganisms and enterotoxin in the food.

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