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Prevalence and Antimicrobial Resistance of *Salmonella* Isolated from Food Handlers in Addis Ababa University Students' Cafeteria, Ethiopia

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Abstract: Diseases spread through food still remain a common and persistent problem resulting in appreciable morbidity and occasional mortality. Food handlers play an important role in ensuring food safety throughout the chain of production, processing, storage and preparation. However, in developing countries like Ethiopia the proportion of certified food handlers and their carrier status is not studied. One of such diseases is salmonellosis that can be transmitted from chronic asymptomatic *salmonella* carriers especially the food handlers. This crossectional study was conducted to determine the *Salmonella* carrier status among food handlers of Addis Ababa university students' cafeteria, Addis Ababa, Ethiopia. A structured questionnaire was used to collect socio-demographic data and risk factors. Stool samples were collected from 233 food handlers for bacteriological techniques (culture, biochemical tests) and drug susceptibility tests were conducted. *Salmonella* species was detected in 8(3.4%) of the examined food-handlers. Two of them were *S. enterica serovar typhi*, one *S. paratyphi* A and the remaining five were other *Salmonella* serovars, interestingly all *Salmonella* positive food handlers were female study subjects. Among the risk factors, hand washing habit after toilet with and without soap had a statistically significant association with carrier status of salmonellae ($p = 0.003$) and odds ratio of 0.07 with a 95% confidence interval (0.008, 0.58). The antimicrobial susceptibility profile showed that all isolates were resistant to ampicillin and all isolates were resistant at least to one of the antimicrobials tested. The finding of 3.4% carriage rate of *Salmonella* species among food handlers could be a source of salmonellosis unless carriers are treated after periodic screening and applying other preventive measures like health education, food handling trainings. Antimicrobial resistance profile also reflects that it would be a serious problem in the near future.

Key words: Carrier · Cross Sectional Study · Food-handler · *Salmonella*

INTRODUCTION

Food-borne diseases are a public health problem in developed and developing countries. The World health organization (WHO) stated that, lots of people suffer from food borne diseases each year, both in developing and developed countries [1]. More than 250 different food-borne diseases have been described. Most of these diseases are infections, caused by a variety of bacteria, viruses and parasites. Other diseases are poisonings, caused by harmful toxins or chemicals like poisonous mushrooms [2].

Bacteria that cause food-borne diseases include among others Salmonella, Campylobacter, *Listeria*, pathogenic Escherichia coli, Yersinia, Shigella and Enterobacter. Salmonellosis is a major bacterial enteric illness in both humans and animals. It constitutes a major public health burden and represents a significant cost to society in many countries. *Salmonella enterica* has more than 2,000 serovars with *S*. enteric serovar Typhimurium and *S. enterica* serovar Enteritidis most commonly encountered globally [3-5].

Salmonella infections can be typhoidal or non-typhoidal. Serotypes such as *S. enterica* serovar *Typhi*,

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S. enterica serovar *Paratyphi A* and *B,* causes of typhoidal salmonellosis, are highly adapted to humans and do not cause disease in non-human hosts. The vast majority of *S. enterica* serovars (e.g., Choleraesuis and Enteritidis), however, are chiefly pathogenic in animals that constitute the reservoir for human infection: poultry, pigs, rodents, cattle, pets (From turtles to parrots) and many others [3].

Typhoid fever is a global health problem. Its real impact is difficult to estimate because the clinical picture is confused with those of many other febrile infections. Additionally, the disease is underestimated because there are little laboratory facilities to efficiently diagnose in most areas of developing countries. These factors are believed to result in many cases going undiagnosed [6]. However, the existing estimate of the global burden of Typhoid fever, serovars Typhi and Paratyphi is 21 million illnesses and 600,000 deaths annually [7]. Typhoid fever (Enteric fever) caused by *Serovar* Typhi is an endemic disease in the tropic and sub-tropic and has become a major public health problem in developing countries of the world with an estimated annual incidence of 540 per 100,000 [6]. The salmonellae that cause Typhoid fever and other enteric fevers spread mainly from person-to-person via the fecal-oral route and have no significant animal reservoirs. Asymptomatic human carriers may spread the disease. Such infections may occur when food or water contaminated by infected food handlers is ingested [3,8].

Although its prevalence varies across regions, diseases caused by *S. enterica* serovars are especially prevalent in developing areas, such as Southeast Asia, Africa and South America that leads to an estimated 20 million cases and 200,000 deaths each year. Challenges such as antibiotic-resistant *Salmonella* strains also pose a significant threat to deliver reliable therapies [6].

In Ethiopia, as in other developing countries, it is difficult to evaluate the burden of salmonellosis because of the limited scope of studies and lack of coordinated epidemiological surveillance systems. In addition, under-reporting of cases and the presence of other diseases considered to be of high priority may have overshadowed the problem of salmonellosis. The real situation of antibiotic resistance is also not clear since Salmonella are not routinely cultured and their resistance to antibiotics cannot be tested. Surveillance for the prevalent *Salmonella* serovars and the assessment of antimicrobial susceptibility is essential to control the spread of the pathogen [9].

Diseases spread through food still remain a common and persistent problems resulting in appreciable morbidity and occasional mortality. Food handlers play an important role in ensuring food safety throughout the chain of production, processing, storage and preparation. However, information is scarce on the proportion of certified food handlers and their carrier for salmonellosis in Ethiopia. Therefore, this study was designed to assess the *Salmonella* carrier status of food handlers and determine the antibiotic pattern of the isolates among food handlers of AAU students' cafeteria, Addis Ababa, Ethiopia.

MATERIALS AND METHODS

The study was conducted among food handlers in Addis Ababa university students' cafeteria from December 2010 up to February 2011, Addis Ababa, Ethiopia.

Study Design and Study Population: A cross sectional study was conducted to assess the carrier status of *Salmonella* among food handlers working in Addis Ababa University students' cafeteria. Study subjects were individuals working as a food-handler in students' cafeteria from different campus of the university. Sample size was determined according to Khurana *et al.* [10].

Since the study population is less than 10,000 that is the total number of food handlers is (N=594) the corrected sample size was 233. In order to get samples, simple random sampling technique was used, after the complete list of food handlers obtained from human resource management office. Inclusion criteria were all food handlers without current clinical symptoms of salmonellosis such as diarrhea, fever, gastroenteritis, vomiting and abdominal cramp. Food handlers excluded from the study included those with clinical signs of typhoid fever and those on antibiotics for at least two weeks prior to the study.

Questionnaire: Participants of the study were interviewed using pre-tested structured questionnaire and it was aimed at gathering information on demographic data (age, sex, educational and service), predisposing factors for salmonellosis such as hygiene (Toilet use, drinking water source, hand washing after toilet use).

Sample Collection and Identification Procedure: Stool samples were collected from 233 apparently healthy food handlers from AAU students' cafeteria. Collected stool samples were transported to the ALIPB laboratory with ice box within 2-4 hours of collection and directly cultured on selenite cystine enrichment broth medium. Isolation of Salmonella species was conducted as Addis *et al*. [11].

Antimicrobial Susceptibility Testing: Each isolate was tested for susceptibility to nine commonly used antimicrobials (ciprofloxacin, chloramphenicol, norfloxacillin, ampicillin, tetracycline, trimethoprem-sulfamethoxazole, ceftriaxone, gentamycin and amoxicillin/ clavulinic acid) using the disk diffusion method according to guidelines set by the National Committee for Clinical Laboratory Standards [12].

Ethical Consideration: Prior to the commencement of this study, ethical approval was obtained from the Ethical Review Committee of the School of Medicine, AAU. Participation of food-handlers was voluntary and written consent was obtained from each subject.

Data Analysis: The data was analyzed using SPSS version 18 computer software. Fisher's exact test results were used to compare different groups and p-value of less than 0.05 was considered as statistically significant.

RESULTS

Risk Factors Associated with Salmonellosis: All of the food-handlers' working at AAU students' cafeteria had a habit of latrine usage and hand washing with and without soap after toilet covering; 65.2 and 34.8%, respectively. In addition, all of them used pipe water as a source of drinking water. Among the 233 food-handlers examined, 62(26.6%) had ever been diagnosed for typhoid fever at different times in their life. Of these, 3(4.8%) food-handlers were carrier for *Salmonella* species in this study, in which two were diagnosed in the last one year and the other one was before fourteen years. Therefore, the carrier rate was higher among those diagnosed than those never been diagnosed for salmonellosis which was 5(2.9%) out of 171 food-handlers. In this study, 158(67.8%) food-handlers have been found to have a history of serological medical checkup inconsistently for typhoidal *Salmonella* organized by the university. However, it has been done before three years in some campuses.

Statistical analysis of medical checkup versus carrier status for *Salmonella* indicated that there is no statistically significant difference in carrier status of *Salmonella* between those medically checked for typhoidal *Salmonella* and not checked ($P>0.05$). Statistical analysis of hand washing habit after toilet showed that there is a statistically significant difference in carrier status of *Salmonella* between food-handlers washed their hands with soap and water and only with water after toilet ($P< 0.05$). Therefore, the chance of getting *Salmonella* infection after washing hands with soap and water is 93% lesser than washing hands only with water after toilet. The odds ratio is 0.07 with a 95% confidence interval (0.008, 0.58).

Salmonella Isolates: Of the 233 food handlers screened, *Salmonellae* were isolated from eight food handlers; giving a *Salmonellae* carriage rate of 3.4%. The serovars of *Salmonella* isolated were *S. enterica* serovar Typhi, *S. enterica* serovar Paratyphi A and other none serotyped *Salmonella* serovars. These isolates were from female food handlers (Table 1).

Antimicrobial Susceptibility Profile of *Salmonella* Isolates: All *Salmonella* species isolated were resistant to ampicillin except one isolate with intermediate susceptibility. Only 2(25%) *Salmonella* isolates (one *S. enterica* serovar Typhi,and one *S. enterica* serovar Paratyphi A) were sensitive to tetracycline while five had intermediate and one resistant result. However, all isolates were sensitive both to ceftriaxone and gentamycine (Table 2).

Of the two *S. enterica* serovar Typhi isolates, one was resistant to chloramphenicol and intermediate to tetracycline while the other one had an intermediate susceptibility to cotrimoxazole and ampicillin. Whereas, the single *S. paratyphi* A isolate had resistance to both ampicillin and amoxicillin+ clavulinic acid. One non- serotyped *Salmonella* isolate was resistant to five antimicrobial agents including ampicillin, ciprofloxacin, cotrimoxazole, norfloxacillin and tetracycline. None of the *Salmonella* isolates identified was sensitive to all antimicrobial discs tested. Therefore, all the isolates were resistant at least to one of the nine antimicrobial agents tested (Table 2).

Table 1: Association of occurrence of *Salmonella* among food handlers of different age, sex, educational background and service year

		Stool culture result for *salmonella*		
Characteristics	No. examined	Positive (%)	Negative (%)	Total No. (%)
Age Group				
18-22	13	1(0.42)	12(5.2)	13(5.62)
23-27	46	2(0.85)	44(18.8)	46(19.65)
28-32	44	2(0.85)	42(18)	44(18.85)
33-37	33	1(0.42)	32(13.7)	33(14.12)
38-42	35	0(0.0)	35(15)	35(15)
43-47	15	0(0.0)	15(6.4)	15(6.4)
48-52	22	1(0.42)	21(9)	22(9.42)
53-57	20	1(0.42)	19(8.1)	20(8.52)
58-60	5	0(0.0)	5(2.2)	5(2.2)
Total	233	8(3.4)	225(96.6)	233(100)
Educational status				
Illiterate	8	0(0.0)	8(3.4)	8(3.4)
Read and write	6	0(0.0)	6(2.6)	6(2.6)
Grade 1-4	17	0(0.0)	17(7.3)	17(7.3)
Grade 5-8	88	5(2.1)	83(35.6)	88(37.8)
Grade 9-12	97	2(0.9)	95(40.8)	97(41.6)
Grade above 12	17	1(0.4)	16(6.9)	17(7.3)
Total	233	8(3.4)	225(96.6)	233(100)
Service year				
<1 year	51	3(5.9)	48(94.1)	51(21.9)
1-2 years	10	0(0.0)	10(100)	10(4.3)
>2 years	172	5(2.9)	167(97.1)	172(73.8)
Total	233	8(3.4)	225(96.6)	233(100)
Sex				
Female	193	8(4.1)	185(95.9)	193(82.8)
Male	40	0(0.0)	40(100)	40(17.2)
Total	233	8(3.4)	225(96.6)	233(100)

Table 2: Susceptibility of isolated *Salmonella* isolates to selected antimicrobial agents

	Salmonella Isolates							
Antimicrobial agent (μg)	ST1	ST2	SP	OS1	OS2	OS3	OS4	OS5
Amoxicillin/ clavulanic acid(30)	S	S	R	R	S	S	S	I
Ampicillin(10)	R	R	R	R	R	I	R	R
Ceftriaxone(30)	S	S	S	S	S	S	S	S
Chloramphenicol(30)	R	S	S	S	S	S	S	S
Ciprofloxacin(5)	S	S	S	S	R	S	S	S
Cotrimoxazole(1.25)	S	I	S	S	R	S	S	S
Gentamycine(10)	S	S	S	S	S	S	S	S
Norfloxacillin(10)	S	S	S	S	R	S	S	S
Tetracycline(30)	I	S	S	I	R	I	I	I

Key: *ST1 = S. typhi isolate 1, ST2 = S. typhi isolate 2, SP = S. paratyphi A, OS1= other salmonella species isolate 1, OS2 = other salmonella species isolate 2, OS3 = other salmonella species isolate 3, OS4 = other salmonella species isolate 4, OS5 = other salmonella species isolate 5, S = Sensitive, I = Intermediate, R = Resistant*

DISCUSSION

This cross sectional study was designed to determine the carriage rate of *salmonellae* among a population of food handlers at AAU students' cafeteria. A carriage rate of 3.4% was determined. Asymptomatic carriage is common in developing countries in which Ethiopia is no exception. This rate of carriage contrasts with a rate of 17.14% among suspected asymptomatic food handlers for *S. enterica* serovar Typhi, in Namakkal, India [13] and in a study from Japan on *Salmonella* carriage rate among food workers in hotels, supermarket, food factories and restaurants; only 0.032% of the faecal samples harbored *Salmonella* and the most common serovars were Agona, Corvallis, Infantis and Enteritidis [14]. Furthermore, none of the food-handlers was positive for *Salmonella* species in a study done on food-handlers working in the cafeterias of the University of Gondar and the Gondar Teachers Training College, Gondar, Ethiopia [15].

However, it is interestingly similar to a study by Feglo *et al.* [16] with Salmonellae carriage rate of 2.3% among food vendors in Kumasi, Ghana, in which three of the six *Salmonellae* isolated, were *S. typhi* and the other three, non-typhoidal Salmonellae species, like in our study: two serovar *Typhi*, one serovar *paratyphi* A and five non-typhoidal *salmonellae* among eight isolates. And a study from China by Show *et al.* [17] who found that 1.83 % of Chinese army recruits and cookers were carriers of *Salmonella* species such as group B, C1, D and E.

We found that carriage rate of serovar Typhi among food handlers was 0.85%. This finding disagrees with a study done by Abera *et al.* [18] in Bahir Dar town, North West Ethiopia, 1.6% food handlers were found infected with serovar Typhi. This may be due to the fact that food handlers in AAU had a better habit of hand washing after toilet, after touching materials and before food handling and also more food handlers were trained in food handling and preparation than food handlers working in the restaurants, cafeterias and hotels of Bahir Dar town. However, it coincides with a study from Amritsar, India by Mohan *et al.* [19], only (0.47%) was found to have serovar Typhi in the stool samples.

The chronic *Salmonella* carrier state occurs most commonly among middle age women [20]. In our study, all asymptomatic *Salmonella* carrier food handlers were females as previous studies in Ghana done by Feglo *et al.* [16], all salmonella positives were females and in Namakkal, India, by Senthilkumar and Prabakaran [13], four out of six Typhi carriers were females. However, the small number of organisms isolated does not permit any reliable conclusions on the carrier status of both sexes.

Many factors may contribute to the difference in the prevalence of *Salmonella* carriage rate among asymptomatic carriers at different times, places and conditions as well as in different population. The possible factors that favor the transmission and prevalence of salmonellosis may include environmental and personal sanitation, socio-economic and living standards, microbial quality, availability of water supply and awareness of safe food handling and preparation among individuals. In this study, most of food-handlers working in students' cafeteria of AAU were females, young adults and had low educational levels; which is in line with a study from Bahir Dar [18] and Gondar town [15].

It is expected that all food-handlers at university, military, hospitals etc cafeterias to have a medical checkup for food-borne pathogens. Despite this fact, the interview result of our study showed that only 67.8% of food-handlers working in AAU students' cafeteria had a medical checkup for typhoidal *Salmonella*. However, in Bahir Dar town, Abera *et al.*[18] found that none of the food-handlers had a medical checkup.

Hygienic assessment of the food-handlers revealed that they had a good habit of hand washing after toilet, touching dirty materials and before food handling. Hand washing habit after toilet with and without soap had a strong association with the carrier status of *Salmonellae* ($p=0.003$) and odds ratio of 0.07 with 95% confidence interval: (0.008, 0.58). The result indicated that 65.2% of food-handlers had a habit of hand washing with soap after toilet while 34.8% washed only with water. This is in parallel with a study by Smith *et al.* [21] in Lagos, Nigeria, 71.8% washed their hands with soap and water while 28.2% washed their hands with only water after visiting toilets. However, a study from Gondar university by Andargie *et al.* [15] and Bahir Dar town by Abera *et al.* [18] found that 89 and 90.6% of food-handlers had a habit of hand washing after toilet respectively. Around 85% of food-handlers had a trimmed finger nail but it had no association with the carrier status of *Salmonella.* Finger nails serve as a vehicle for transport microorganisms from their source to the foods or/and directly in to the body.

Inadequate or absence of treatment and asymptomatic cases of salmonellosis directly contribute in the increment of *Salmonella* carriers. Therefore, carriage of *Salmonella* following inadequate treatment

may increase the probability of carrying a drug resistant strain. Even though no MDR *Salmonella* was detected, this study indicated that there is an increase in antimicrobial resistance of *salmonellae* isolated from carriers especially to ampicillin and tetracycline. Seven of eight *Salmonellae* isolated (87.5%) were resistant to Ampicillin, where one was intermediate. It agrees with a finding by Abera *et al.* [18] from Bahir Dar, Ethiopia and Valli *et al.* [22] from Tamil nadu, India, where 100% of isolates were resistant to ampicillin. However, it differs from a result found by Mache [23] from Jimma, Ethiopia; only 54% were resistant to ampicillin.

Ceftriaxone and gentamycin and fluoroquinolones were highly effective against all isolates. Ciprofloxacin is the drug of choice for the treatment of salmonellosis and carrier state according to the current guidelines for the treatment of salmonellosis. However, one of the isolates in this study showed resistance to ciprofloxacin. One of two Typhi isolates showed resistance only to ampicillin and chloramphenicol which is contrary to a study by Senthilkumar and Prabakaran [13] from Namakkal, India; five out of six Typhi isolates were multi-drug resistant. Furthermore, a study by Abera *et al.* [18] from Bahir Dar Typhi isolates showed resistance to Ampicillin, Cotrimoxazole, tetracycline and chloramphenicol, genentamicin and Norfloxacillin. These differences might be due to low number of isolates in our study.

In conclusion, Food-handlers in AAU students' cafeteria had a good habit of hand washing with or without soap. It was found that washing hands with soap reduces the chance of *Salmonellae* carriage rate by 93% than washing only with water ($p = 0.003$). However, the 3.4% carriage rate of *Salmonella* species among food-handlers serving in AAU students' cafeteria can be a possible source of salmonellosis for the students unless carriers treated or other preventive measures taken. This study showed that all of the *Salmonellae* isolates had a resistance at least to one of the antimicrobials tested. Increasing antimicrobial resistance of *Salmonella* with no doubt may result in higher death to case ratios for resistant *Salmonella* infections than for infections with sensitive strains.

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