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RESEARCH ARTICLE

PREVALENCE OF *SALMONELLA* AND INTESTINAL PARASITES AMONG FOOD HANDLERS PREDISPOSE CONSUMERS TO SIGNIFICANT HEALTH RISKS

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Abstract

Background and objectives: Food borne diseases are a global public health problem and food handlers play a major role for the transmission of food borne diseases. This study was aimed at exploring the prevalence of intestinal parasites, *Salmonella typhi* carrier rate and risk factors of infection with typhoid and/or intestinal parasites among food handlers at Ibb city, Yemen.
Subjects and methods: A cross sectional survey was conducted among three hundred and fifteen food handlers, in age ranges from 14 to 65 years. All individuals were working in restaurants, cafeterias or school buffets in Ibb city. For collecting data; a pre-tested structured questionnaire was used. Stool samples were examined for intestinal parasites microscopy and for *S. typhi* by stool culture media and blood for detection antibodies per the standard laboratory methods were used.
Results: A total of 315 food handlers in Ibb city over a 12-month period were enrolled in this study, ages ranged from 14 to 65 years, with a mean±SD age of 31.2±11.9 years. The highest prevalence of antibodies against *S. typhi* antigen suspension O was 18.4%, while antibodies against *S. typhi H* antigen suspension were 7.6%. Also, the positive rate for total *S. typhi* antibodies ELISA IgG was 9.5% and the positive rate for *S. typhi* stool cultures was 7.3%. The overall prevalence of intestinal protozoa was 20%, the most intestinal parasitic prevalent was *Entamoeba histolytica* (15.6%), followed by *Ascaris lumbricoides* (12.1%), *Hymenolepis nana* (4.4%), and *Schistosoma mansoni* (3.2%).
Conclusion: Inexperienced and poor personal hygienic food handlers play a role in the transmission of food-borne infections. Local health authorities should implement food handlers training on food safety, institute periodic focused medical check-up for food handlers and improve human waste disposal.
Keywords: Food handlers, Intestinal parasites, Ibb city, *S. typhi*, Yemen.

INTRODUCTION

Food-borne diseases are a public health problem in developing and developed countries. The World health organization (WHO) predictable that up to 30% of the population in developed countries, suffer from food-borne diseases each year, while up to 2 million deaths in developing countries are estimated per year[1]. In developing countries such as Yemen, intestinal parasitic infections are public health problems. Studies illustrated that parasitic infections of intestine consequence in morbidity, mortality, malnutrition, and socioeconomic impact owing to treatment expenditure and hospitalization cost[2,3]. Intestinal parasites, which have a direct life cycle, are transmitted by the faecal-oral route to humans because of poor personal hygiene[4]. *Salmonella typhi* is one of the major causes of food and water-borne gastroenteritis in humans[5] and remains an important health problem in Yemen and worldwide.

The WHO approximates 16 million recent cases and 600,000 deaths of typhoid fever were expected each year[1]. The appearance of antimicrobial resistant *S. typhi* including to chloramphenicol has been an issue[4,6]. Carriage of *S. typhi* asymptomatically amongst food handler with poor personal hygiene and deficient knowledge of food safety could be the resource of food-borne pathogens[1]. The outcome of food

contamination fluctuates amongst regions and countries of the world depending on geography, climate and degree of social and economic development[1,2]. The WHO's Department of Food Safety and Zoonoses (FOS) provides scientific advice to organizations and the public on issues related to food safety. Its mission is to reduce the burden of foodborne diseases, thereby promoting health security and sustainable development of member states. WHO is working closely with the Food and Agriculture Organization of the United Nations (FAO) to address food safety issues along the entire food production chain from production to consumption using new methods of risk analysis. These approaches provide effective, science-based tools for improving food safety, thus benefiting both public health and economic development[7].

In Ibb city, Yemen, drinking and eating in food service businesses, such as restaurants, hotels and snack shop is turn out to be a common practice. Data on *S. typhi*, intestinal parasites and risk factors among food-handlers in the study area is inadequate. Therefore, this study aimed to determine the prevalence of intestinal parasites, *S. typhi* and explore risk factors among food handlers working in food service establishments in Ibb city, Yemen.

SUBJECTS AND METHODS

Study population: This cross sectional study was carried out during a period of one year, starting in 1-2-2019 and ending in 1-2-2020. Three hundred and fifteen food handlers were included, in age groups ranging from 14 to 65 years. All individuals were working in restaurants, cafeterias or school buffets in Ibb city.

Sample size: The sample size was calculated in Epi Info 6 version 6.04 taking into consideration the following: The size samples of the population were 5000. The expected frequency of the factor was 5%. If 5% is the true rate in the population and the worst acceptable percent is 1%, with confidence level of 99%, the sample size would be not less than 302 selected individuals. The number increased to 315 to have more precise results.

Data collection: A full history of risk factors of contracting infections among food handlers and their demographic data were taken from each studied individual; and the findings were recorded in a predesigned questionnaire. The data collected included name, age at the time of the study, sex, residence, occupational status, and personal hygiene practices, history of typhoid, intestinal protozoa infections and intestinal parasitic infections etc. Also laboratory results of stool investigations, stool culture and ELISA IgG for typhoid were included in this questionnaire.

Collection and transferring stool samples: Stool specimens were collected from food handlers in Ibb city. Specimens were collected in sterile screw capped containers. Then prepared for microscopic examination and bacteriological culturing.

Microscopically: Each fresh sample were examined microscopically for cysts and Trophozoites of *Giardia lamblia* and *Entamoeba histolytica* by using a saline and trachoma stain and investigated samples by concentration method for intestinal helminthes and cysts of *E. histolytica*, and *G. lamblia*[8].

Isolation of Pathogenic Bacteria: All samples were cultured in different selective media such as; MacConkey sorbitol agar, xylose lysine deoxycholate agar (XLD), and selenite broth. Plates were incubated for 18 hours at 37°C aerobically, the selenite broth then subculture onto *Salmonella-Shigella* agar (S.S agar)[8].

Identification of Isolated Bacteria: Colonies had been identified based on morphologic characteristics and other standard Biochemical reaction, Kligler Iron Agar (KIA), Motility Indol Urea (MIU) and Oxidase tests are recommended to differentiae Species of bacteria or to identify them[8].

Detection of *Salmonella* spp (pathogenic strains): Isolated *Salmonella* spp were examined by *Salmonella* Vi Antisera.

Widal test: The food handler's serum was tested for O and H antibodies (agglutinins) against the following antigen suspensions (stained suspensions): (antibodies titer higher than 1/80): *S. typhi* 0 antigen suspension (9, 12), *S. typhi* H antigen suspension (d), *S. paratyphoid* A O antigen suspension (1, 2, 12); *S. paratyphoid* A H antigen suspension a.

ELISA IgG for typhoid: Total anti-bodies IgG against typhoid and paratyphoid quantitative were determined by an Enzyme-linked immunosorbent assay (ELISA) using a commercially available kit provided by Biokit, Spain.

Ethical consideration: Consent was taken from all the participants and the participants were informed that participation is voluntary and that they can refuse without giving any reason.

Statistical analysis: The data were analyses performing Epi Info statistical program version 6 (CDC, Atlanta, USA). Conveying the quantitative data like mean values, standard deviation (SD), as the data were normally distributed. The qualitative data were expressed as percentages; for comparison of two variables to determine the *p* value, the Chi square test was used. Odd ratio (OR) was used with 99% confidence interval. The *p* value <0.05 was regarded as statistically significant.

RESULTS

The tested food handlers ages were ranged from 14 to 65 years old, most of individuals were in age groups of 20-29 years (40 %), followed by age group 30-39 years (30.2%). The mean age ±SD for the tested food handlers was 31.2 years±11.9 years (Table1). Most of individuals had primary school level (46.3%), followed by illiterate level (30.2%), but secondary level and higher were only 13% and 10.5% respectively (Table 1). The prevalence of *S. typhi* positive stool culture was 7.3% (Table 2). The highest prevalence of antibodies against *S. Typhi O* antigen suspension 9, 12 was 18.4%, while antibodies against *S. typhi* H antigen suspension, d were only 7.6%. Also the prevalence of antibodies against *S. paratyphoid* A O antigen suspension, 1, 2, 12 and *S. paratyphoid* A H antigen suspension were 7.6% and 6.3% respectively.

Table 1: Scio-demographic characterization of participants.

Variable	Category	No. examined	Rate %
Age groups (in years)	< 20 years	31	9.8
	20-29 years	126	40
	30 -39 years	95	30.2
	≥ 40 years	63	20
	Statistical	Mean age= 31.2 years	SD= 11.9 years
		Max =65 years	Min=14 years
Educational level	Illiterate	95	30.2
	Primary School	146	46.3
	Secondary	41	13
	Higher	33	10.5

The positive rate of ELISA IgG total *S. typhi* antibodies was 9.5% and the positive rate of stool culture *S. typhi* was 7.3% (Table 3). The total prevalence of intestinal protozoa was 20%; *E. histolytica* was 15.6%, in and *G. lamblia* was 4.4% (Trophozoites was 3.5%, Cysts was 4.4%) (Table 4). The total prevalence of intestinal parasites was 19.7%; *Ascaris lumbricoides* was 12.1%, *Hymenolepis nana* (4.4%), and *Schistosoma mansoni* (3.2%) (Table 5). In hand washing practices, 210 (66.7%) food handlers had a habit of hand washing by water only after toilet. However only 60 (19%) food handlers had a habit of hand washing by water and soap after toilet and 45 (14.3%) of the food handlers have the habit of not washing hand after the toilet (Table 6). However, a less number (49.8%) of food handlers had a habit of hand washing after touching dirty materials and different body parts (hair, nose and ear) between handling of food items. Only 31(9.8%) of the participants had had medical checkup including stool examination previously.

Table 2: Prevalence of intestinal *S. typhi* and *Shigella* species among study subjects.

Bacteria	Frequency Number (%)
S. typhi positive culture	23 (7.3)
Shigella species positive culture	3 (0.95)

Table 3: Frequency of *S. typhoid* antibodies among food handlers.

Markers	Frequency Number (%)
Widal test (antibodies titer higher than 1/80)	
S. Typhi O antigen suspension 9,12	58/315 (18.4)
S. Typhi H antigen suspension, d	24/315 (7.6)
S. Paratyphoid A O antigen suspension, 1, 2, 12	24/315 (7.6)
S. Paratyphoid A H antigen suspension, a	20/315 (6.3)
ELISA IgG total *S. typhi* antibodies	30/315 (9.5)
Positive stool culture for *S. typhi*	23/315 (7.3)

Two hundred and ten (66.7%) food handlers were certified for training in food handling and preparation (Table 6). When we considered sources of water use in the restraints 80% of sites were used tape water, while 20% of the sites were using tank water. 60% of food handlers were wearing special food clothes and 40% not practices that 79% of participants using reuse plastic tools. There were only 6 (1.9%) food handlers had past history of typhoid (Table 7).

Table 4: Prevalence of intestinal protozoa among studied food handlers.

Protozoa species	Frequency Number (%)
E. histolytica	49/315 (15.6)
Trophozoites	4/315 (1.3)
Cysts	48/315 (15.2)
G. lamblia	14/315 (4.4)
Trophozoites	11/315 (3.5)
Cysts	14/315 (4.4)
Total	63/315 (20)

DISCUSSION

The current study established the *Salmonellae* carriage among a population of food-handlers in Ibb city, Yemen was 7.1%. This high rate differs with the rate of 0.13% approximation for the developed world[9,10], and is similar to the rate of 6.5% of Kumalo *et al.*,[11] in Ethiopia recently. Yet others; Gelosa *et al.*, in Italy[13], and Yamada *et al.*, in Tokyo[14] have reported only 1.68% and 0.7% respectively.

High prevalence of carriage intestinal *S. typhi* in the current study is attributed by poor environmental sanitation, poor personal hygienic practices and absent of policy regulates food safety. The current result also confirmed the finding of Tsen *et al.*,[15] and Turki *et al.*,[16] in which they found that *S. typhi* is one of the major causes of food and water borne gastroenteritis in human and remains an important health problem worldwide. Studies had demonstrated that food handlers harbor *S. typhi* asymptomatically[9,11].

Table 5: Prevalence of intestinal parasites (helminthes) among study subjects.

Parasites species	Frequency Number (%)
Ascaris lumbricoides	38/315 (12.1)
Hymenolepis nana	14/315 (4.4)
Schistosoma mansoni	10/315 (3.2)
Total	62 (19.7)

The high rate of carriage of *S. typhi* might lead to outbreak of typhoid in Ibb city, a report from Spain[18] wherever one chronic carrier, an accidental food-handler, was revealed to have infected 70 others, still highlights the continued importance of chronic *Salmonellae* carriers especially food handlers in the spread of the disease a fact that has been long established[11,12,14,19]. An attempt was also made through this study to establish the *Salmonella* carriage among a group of food handlers in the city of Ibb, Yemen by Widal test; 18.4% has been identified. Regardless of the constraint, the obtained value is thus a partial reflection of the expected total[20]. In the publication, Frimpong *et al.*,[20] proposed to be adopted for the diagnosis of enteric fever, Widal titer 1/160 and 1/320 for anti-O and anti-H, respectively. Remarkably, food handlers with positive stool cultures of non- typhoidal *Salmonellae* had a low titer.

Table 6: Frequency of hygienic practices of food handlers.

Variable	Category	No. examined	Rate %
Certified in food training	Yes	210	66.7
	No	105	33.3
Hand washing after toilet by water only	Yes	189	60
	No	126	40
Hand washing after toilet by soap	Yes	60	19
	No	252	81
Hand washing after touching dirty materials	Yes	157	49.8
	No	158	50.2
Touching body parts during food handling	Yes	221	70.2
	No	94	29.8
Medical check up	Yes	31	9.8
	No	284	90.2

A rate of 9.5% (Table 3) was determined by ELISA for typhoid. The previous method as Widal test of using an antibody detection assay was recently introduced in favor of using stool cultures and an ELISA assay, although only with limited differentiation of carriers and prior infection[21]. So the method used is appropriate. Regardless of the constraint, the obtained value is thus a partial reflection of the expected total[21]. In the results of the current study, it can be suggested that all food handlers who had a positive ELISA IgG antibody were carriers of the causative bacteria or that they had enteric fever, since all of them were culture positive for *S. typhi*. In this study, the total prevalence of intestinal protozoa was 20%. The prevalence of intestinal *E. histolytica* was 15.6%, in which Trophozoites was seen in 1.3% only and cyst was seen in 15.2% (Table 4). However, a low prevalence of *G. lamblia* present among food handlers in which it was 4.4%.

Table 7: Associated risk factors with bacterial and protozoa transmission among food handlers.

Variable	Category	No. examined	Rate %
Source of water	Tape water	252	80
	Tank water	63	20
Wearing food clothes	Yes	189	60
	No =126	126	40
Reuse plastic tools	Yes	249	79
	No	66	21
Past history of typhoid	Yes	6	1.9

The overall prevalence of intestinal protozoa among food handlers in the current study was similar compared to previous study done at Gondar town (20.1%) in North West Ethiopia[22], and Kumalo *et al.*, in Dawuro Zone (20.4%), South-Western Ethiopia[11] but significantly higher than that reported by Davoud *et al.*, in Iran in which about 4% food handlers had intestinal *protozoa*[23]. High prevalence of intestinal protozoa is attributed by poor environmental sanitation and deprived personal hygienic practices. Active Trophozoites forms of *G. lamblia* and E. *histolytica* were associated with diarrheic food handlers. *G. lamblia* infected food-handlers can directly spread to consumers if ingested *via* contaminated food and contaminated water because *G. lamblia* cysts does not need environmental maturation[8]. Furthermore, Mintz *et al.*, established that food handlers infected with *G. lamblia* were a vehicle for *Giardia* outbreak in commercial food establishment [24]. Therefore, food handlers should be in a good health and those suffering from diarrhea must be excluded from work until they have been completely free of symptoms after treatment. In this study, most food handlers working in the kitchens were very young adults in age groups of 20-29 years (40 %), (Table 1) and the majority had inexperienced with low educational levels, in which

most of individuals had only primary school level (46.3 %), or illiterate (30.2 %) (Table 1), which agrees with previous studies in developing countries[11,16,25].

In the current study only 31(9.8%) of the participants had had medical checkup including stool examination previously. Two hundred and ten (66.7%) food handlers were certified for training in food handling and preparation. However, in developing countries in Africa and Asia from 22.7% to 46% of the food handlers had medical checkup including stool examination in the past[11,25,26]. Assessment of hand washing practices revealed varied results in current study 210 (66.7%) food handlers had a habit of hand washing by water only after toilet. However only 60 (19%) food handlers had a habit of hand washing by water and soap after toilet and the rest had not washing by water or soap after toilet. The current results were in parallel with the previous reports in Ethiopia and India[11,22,26]. In spite of this, fewer hands-washing practices after touching dirty soiled items and different body parts were in between handling food items[27-34]. This revealed that food handlers lack awareness about food contamination due to poor hygiene practices. Health education intervention on food safety and hygiene should be strengthened to ensure food safety during processing, preparation and storage in food service establishments[35-40].

Limitation of the study

Despite the many recent studies that discussed food borne diseases, gastrointestinal infections, bacterial infections and protozoa in the digestive system in Yemen[26-39], the current study did not include other bacterial and viral infections that may be transmitted from workers in restaurants to customers from community members, and therefore these shortcomings must be taken into account in this study. We recommend that different techniques be evaluated more systematically to study this health problem and include infectious pathogens that have been performed among other populations previously in Yemen and performed among food handlers.

CONCLUSIONS

In conclusion, *S. typhi* intestinal carriage rate, the intestinal protozoa infections and intestinal parasitic infection rates of food handlers of Ibb city were relatively high. The findings emphasize that food handlers with different pathogenic microorganisms may predispose consumers to significant health risks. Therefore, constant epidemiological surveillance through biannual routine parasitological tests and treatment of the infected cases along with the improvement of environmental sanitation is recommended to control *S. typhi*, the intestinal protozoa infections and intestinal parasitic infection in food handlers in Ibb city. Inexperienced and poor personal hygienic food handlers play a role in the transmission of food-borne infections. Local health authorities should implement food handlers training on food safety, institute periodic focused medical check-up for food handlers and improve human waste disposal.

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AUTHOR CONTRIBUTIONS

This research is part of a master's degree in the Department of Medical Microbiology, Faculty of Medicine and Health Sciences, Sana'a University. **Al-Ghaithi GAAA:** field work, laboratory work. **Al-Moyed KA:** methodology, investigation. **Al-Shamahy HA:** supervision, review. **Al-Haddad MA:** writing, review, and editing, methodology. All the authors approved the finished version of the manuscript.

DATA AVAILABILITY

The datasets generated during this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

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