

Detection of *Salmonella* carriers among apparently healthy cafeteria food handlers in tertiary care centre Pokhara

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ABSTRACT

Introduction: Foodborne illnesses are caused by consumption of contaminated foods. The risk of food getting contaminated depends largely on the health status of the food handlers, their personal hygiene, knowledge and practice of food hygiene. Hence, this study aimed to assess the proportion of *Salmonella* among food handlers working in the cafeteria of Gandaki Medical College Teaching Hospital, Pokhara, Nepal. **Methods:** An institutional based cross-sectional analytical study was conducted from March 2018 to February 2019. A structured questionnaire was used to collect the sociodemographic characteristics, the knowledge and the risk factors of the participants. A total of 62 stool samples were collected and processed according to the standard microbiological procedures. All the *Salmonella* isolates were identified by biochemical tests. Additionally, antimicrobial susceptibility tests were performed according to Clinical and Laboratory Standards Institute guidelines 2017, using Kirby-Baur disk diffusion method. Data were analyzed by Statistical Package for Social Service for window version 21.0. Descriptive statistics were computed and chi-square test was applied at 5% level of significance. P-value <0.05 was considered to be statistically significant. **Results:** Total of 62 food handlers were included in the study. Majority of the cafeteria workers 46(74.2%) were not aware of proper hand washing technique. Of the 62 food handlers working in the cafeteria, *Salmonella* were isolated from 12(19.4%) stool specimens. All the *Salmonella* isolates were sensitive to amikacin (100%), followed by imipenem (91.7%) and cefotaxime (91.7%). However, the highest rate of antibiotic resistance among *Salmonella* isolates was noted for amoxicillin (58.3%). **Conclusions:** This study showed increased proportion of *Salmonella* carriers among the food handlers of cafeteria. *Salmonella* carriage among food handlers is of great concern as they can be the source of foodborne illness. Therefore, educating the food handlers of cafeteria about personnel hygiene may play a pivotal role in minimizing the incidence of foodborne illness and the associated morbidity.

Keywords: Antibigram, food handlers, personnel hygiene, *Salmonella*.

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INTRODUCTION

Foodborne illnesses are caused by consumption of contaminated foods by both infectious (viral, bacterial, fungal and parasitic) and non-infectious toxins, chemicals, physical agents.¹ The risk of food getting contaminated depends largely on the health status of the food handlers, their personal hygiene, knowledge and practice of food hygiene.² Although foodborne diseases have significant global health burden, these infectious diseases can be preventable if public health measures are followed to prevent contamination of food during its production, processing, distribution and preparation.³

A report from Foodborne Disease Burden Epidemiology Reference Group (FERG) mentioned that South-east Asia Region recorded more than 150 million illnesses and 175,000 deaths by food borne diseases in 2016.⁴ Of the bacterial agents *Salmonella*, *Shigella*, *Listeria*, *Escherichia coli*, *Campylobacter* are the major pathogens responsible for foodborne illness.⁵⁻⁷, *Salmonella* species is one of the

major causes of foodborne gastroenteritis in human, and remains an important public health problem with 25 million annual incidences and more than 200000 associated deaths globally.⁸ The main cause of Salmonellosis is asymptomatic carriers. An individual can asymptotically carry *Salmonellae* for years without showing any of the symptoms of *Salmonella* infection. In such carriers, *Salmonellae* continue to multiply in the gall bladder and reach the intestine through the bile duct and are excreted out in the feces. In case of poor hygienic practices, these bacterial agents reside in the skin and finger nails of the food handlers and get transmitted to the food they process and prepare. So, as to cause food borne Salmonellosis in food consumers.⁹

Nepal, being an underdeveloped country, has a high burden of foodborne illness, mainly because of lack of proper knowledge of personal hygiene and sanitations among food handlers. Herein, we investigated proportion of *Salmonella* carriers among food handlers working in the cafeteria of Gandaki Medical College Teaching Hospital (GMCTH), Pokhara, Nepal. Moreover, antibiotic sensitivity pattern of the isolates were also determined.

METHODS

Study design and period

The study was a cross-sectional analytical study conducted from March 2018 to February 2019. The study was conducted on all the cafeteria workers of Gandaki Medical College and Teaching Hospital (GMCTH) Pokhara, Nepal.

Study population and size

All the individuals working in the cafeteria of GMCTH were included in the study. Convenient sampling method was used to collect the clinical samples and sample size was calculated by using following formula,

$$\begin{aligned} n &= z^2 \times (p \times q) / e^2 \\ &= (1.96)^2 \times (0.091 \times 0.90) / 0.08^2 \\ &= 49.14 \end{aligned}$$

Sample size = 50

Where,

n = required sample size

p = prevalence of study¹⁸, 9.1%

q = 1-p

e = margin of error, 8%

z = 1.96 at 95% confidence interval

All food handlers working in GMCTH's cafeterias were included in the study. Food handlers who had recent history of fever, diarrhea, antibiotic therapy and who did not provide consent were excluded from the study.

Data Collection Tool and Procedure

A structured questionnaire was used for data collection on sociodemographic characteristics (gender, age and educational status) and associated risk factors (nail growing habit, knowledge of hand washing technique and use of gloves while handling food).

Sample Collection and Processing

Stool specimen was collected in sterile wide mouthed container from the food handlers working in the cafeterias of GMCTHRC. The specimens were transported from the collection site to the laboratory in ice bag. Stool specimens were inoculated in Selenite F broth and incubated for six hours at 37°C followed by subculture on Salmonella-Shigella (SS) agar at 37°C for 24 hours. In case of positive growth, bacterial smear was prepared from colonies and stained by Gram staining technique to find gram negative organism. Identification of bacteria was done based on the colony morphology and change in the physical appearance of the differential media and by using standard biochemical tests used for identification of *Salmonella*.

Antimicrobial susceptibility testing

Antimicrobial susceptibility pattern of the isolated *Salmonella* was performed by Kirby Bauer disc diffusion method as per the Clinical and Laboratory Standards Institute (CLSI) recommendations. Briefly, colonies were inoculated in sterile peptone water and incubated to reach the concentration of 0.5 McFarland standards. Sterile swab was dipped into the suspension, excess fluid was squeezed out and the swab was spread over the Muller-Hinton agar plate. Antimicrobial discs were placed onto the media and incubated at 37°C for 18 hours. All *Salmonella* isolates were tested against antibiotic disks (Hi-Media); amikacin (30 µg), amoxicillin (30 µg), cefixime (5 µg), cefpodoxime (5 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), imipenem (10 µg) and ofloxacin (5 µg). The zone of inhibition was measured in millimeter for each disc using sliding caliper and was compared with comparative chart provided by the manufacturer. Results were reported as sensitive or resistant to the antimicrobial agents according to CLSI guidelines 2017.¹⁰

Ethical considerations

Ethical clearance was obtained from Institutional Ethical Committee (reference no. 07-11-074). Informed consent

was taken from all the food handlers participated in the study.

Statistical Analysis

All the data were entered in MS Excel (Microsoft office 2007) and then analyzed by Statistical Package for Social Service (SPSS) for window version 21.0. Descriptive statistics were computed and Chi-square test was applied at 5% level of significance. P-value <0.05 was considered to be statistically significant.

RESULTS

Characteristics of study population and *Salmonella* carrier status

Among the total 62 food handlers participated in the study; 32(1.6 %) were male and 30/62(48.4%) were females. *Salmonella* species were isolated from 12 out of 62 stool specimens. The age of participants in the study ranges between 20 to 60 years. Majority of the food handlers working in the cafeteria were in the age range of 20 to 40 years 46 (74.2%). Majority of the food handlers are literate holding either primary level 32(51.6%) or secondary level 20(32.2%) or with higher level 4(6.4%) of education as shown in Table 1.

Table 1: Characteristics of the study population and *Salmonella* carrier status

Characteristics	Number	Percent (%)
Gender		
Male	32	51.6
Female	30	48.4
Age group		
20-40 years	46	74.2
41-60 years	16	25.8
Education Level		
Illiterate	6	9.7
Primary	32	51.6
Secondary	20	32.2
Higher	4	6.4
Training – Certificate of Food Handling		
Trained	12	19.4
Untrained	50	80.6
<i>Salmonella</i> carrier		
Positive	12	19.4
Negative	50	80.6
Knowledge of Hand washing Technique		
Yes	16	25.8
No	46	74.2
Use Gloves while Handling Food		
Yes	2	3.2
No	60	96.8
Nail Growing Habit		
Yes	20	32.3
No	42	67.7

In this study 12(19.4%) food handlers had certificate of

food training. However, majority of them did not take food handling training 50(80.6%). Although majority did not have habit of nail growing 42(67.7%), they did not have knowledge of proper hand washing technique 46(74.2%). Among the participants, majority of the cafeteria workers did not use gloves during food handling 60 (96.8%).

Of the 62 food handlers working in the cafeteria, *Salmonella* were isolated from 12(19.4%) stool specimens in the study population. Among the *Salmonella* carriers 6/32(18.8%) were male and 6/30(20%) were females. Chi square analysis did not show association between *Salmonella* carrier status and gender (p=0.901) as shown in Table 2.

Table 2: Distribution of *Salmonella* carrier across socio-demographic variables

	Salmonella carrier		Chi-square value	P- value
	Positive n(%)	Negative n (%)		
Gender				
Male	6 (18.8)	26 (81.3)	0.016	0.901
Female	6 (20.0)	24 (80.0)		
Age group				
20-40 years	10 (21.7)	36 (78.3)	0.714*	0.343
41-60 years	2 (12.5)	14 (87.5)		
Training -Certificate of Food processing				
Trained	3 (25.0)	9 (75.0)	0.686*	0.422
Untrained	9 (18.0)	41 (82.0)		

* Fisher’s Exact Test

Distribution of *Salmonella* carrier across behavioral variables

Among the total *Salmonella* carrier positive food handlers 6(30%) had the habit of nail growing, 2 (12.5%) had the knowledge of hand washing technique and 1(50.0%) used gloves while handling food as shown in Table 3.

Table 3: Distribution of *Salmonella* carrier across behavioral variables.

	Salmonella-carrier		Chi-square value	P- value
	Positive n(%)	Negative n(%)		
Nail Growing Habit				
Yes	6 (30.0)	14 (70.0)	2.143	0.143
No	6 (14.3)	36 (85.7)		
Knowledge of Hand washing Technique - six steps				
Yes	2 (12.5)	14 (87.5)	0.714*	0.343
No	10 (21.7)	36 (78.3)		
Use of Gloves while Handling Food				
Yes	1 (50.0)	1 (50.0)	0.352*	0.352
No	11 (18.3)	49 (81.7)		

* Fisher’s Exact Test

Antibiogram of *Salmonella* species

The antibiotic sensitivity of the isolated *Salmonellae* were tested against the eight antibiotics as shown in Table 4.

We found that all of the isolates were sensitive to amikacin. Most of the isolates showed variable sensitivity pattern against the antibiotics used; 11/12(91.7%) isolates were sensitive to cefotaxime and imipenem, 10/12(83.3%) were sensitive to ofloxacin ciprofloxacin. Whereas, 9/12(75.0%) and 8/12(66.7%) of sensitivity was shown by cefixime and cefpodoxime respectively. In contrast more than half of the isolates 7/12(58.3%) were resistant to amoxicillin.

Table 4: Antimicrobial patterns of *Salmonella* species isolated from food handlers

Antibiotics (μ g)	Salmonella species			
	Sensitive		Resistant	
	No.	%	No.	%
Amikacin (30)	12	100	0	0
Amoxicillin (30)	5	41.7	7	58.3
Cefixime (5)	9	75.0	3	25.0
Cefpodoxime (5)	8	66.7	4	33.3
Cefotaxime (30)	11	91.7	1	8.3
Ciprofloxacin (5)	10	83.3	2	16.7
Imipenem (10)	11	91.7	1	8.3
Ofloxacin (5)	10	83.3	2	16.7

DISCUSSION

Food handlers may carry a wide range of enteropathogens and participated in the transmission of many infections to the public in the community and to patients in hospitals. The spread of disease via food handlers is a common and persistent problem worldwide.¹¹ Herein, we investigated the proportion of *Salmonella* carrier state and the associated risk factors among food handlers working in cafeteria of GMCTHRC.

Our study found that 19.4% of the food handlers were positive for *Salmonella* isolates. The proportion of *Salmonella* (19.4%) among the food handlers was in agreement with the study conducted in Tamil nadu, India (17.1%).¹² However, higher percentage of *Salmonella* was isolated in the studies carried out in Nigeria like Owerri (66.7%),¹³ Karu Local Government Area of Nasarawa State (62.7%)¹⁴ and federal Capital Territory of Nigeria (42.3%).¹⁵ In contrast, several other studies conducted in food handlers working in cafeteria have 1 to 9.1% positivity for *Salmonella* carriage.¹⁶⁻¹⁸ The discrepancy of these bacterial isolates in different places might be due to difference in sample processing method, sample size, epidemiological distribution of pathogens, level of environmental sanitation and personal hygiene.

In this study a high proportion of *Salmonella* carrier was alarming which could be due to lack of proper hand washing knowledge and nail growing habit. Prevalence of

Salmonellosis was significantly associated with untrimmed nail and improper hand washing procedure of food handlers after going to toilet, after touching body and before preparing food.¹⁹ Hence, food handlers with *Salmonella* carrier state should not be allowed to work in cafeteria until they are treated and cured.

In present study, *Salmonella* species was 100% sensitivity to amikacin which is in the line with the studies done in Addis Ababa, Ethiopia 100%¹⁸ and Chitwan, Nepal 100%.²⁰ Antibiotic resistant *Salmonella* species is a global concern; especially resistance to extended spectrum- β -lactamase.²¹ We found that 58.3% of *Salmonella* isolated from the food handlers in our study are resistant to broad spectrum β -lactam antibiotic amoxicillin. However, in a study carried out in Ethiopia, 100% resistance for amoxicillin was reported against *Salmonella* recovered from cafeteria food handlers.¹⁸ Additionally, emergence of multidrug resistance *Salmonella* led the use of quinolones as a therapeutic treatment option. A study conducted in 2003 in Bangladesh has demonstrated 100% sensitivity to ciprofloxacin.²² Again, in 2010 a study conducted in North India showed 91% of *Salmonella* isolates were sensitive to ciprofloxacin.²³ However, the present study showed only 83.3% of the *Salmonella* isolates being sensitive ciprofloxacin and ofloxacin. Our results were not in agreement to the study conducted in cafeteria of Kasturba Medical College, Mangalore, India wherein the *Salmonella* isolates were 63% sensitive to ciprofloxacin.²⁴ These results suggested increasing trend in quinolone resistance which is a treatment option for enteric fever, is quite alarming. This potentially points towards the irrational use of antibiotics in our community.

CONCLUSIONS

The study revealed a high proportion of *Salmonella* carrier in asymptomatic food handlers. This study also showed increased resistance to stool culture and sensitivity to commonly used antibiotics such as amoxicillin and cephalosporin. Hence, there should be rationale use of antibiotics in patients. *Salmonella* carriage among food handlers is of great concern as they can be the source of foodborne illness. Therefore, health education intervention on food safety and hygiene should be strengthened to ensure food safety during its preparation and storage in food service establishments and all the *Salmonella* carriers must be refrained from the work until they are treated and cured.

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