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Original Article

A questionnaire-based study to identify the physiological implications of unpleasant hall dining foods among university students

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ABSTRACT: Most of the residential halls of the public universities in Bangladesh are unable to ensure the hygienic environment for students. Therefore, a pilot study was conducted to evaluate the levels of student's satisfaction regarding the hygiene status, and the quality of offered foods and water in four different halls of Shahjalal University of Science and Technology, Sylhet, Bangladesh. University students (N =100; 50% female and 50% male) from different disciplines with an age range of 18-22 years were surveyed using a fixed structured questionnaire. We observed that most of the students were highly dissatisfied with the hall dining options and the quality of supplied foods and water. Food and water samples were collected from these four different hall dinings to evaluate the presence of pathogenic bacteria and their antibiotic susceptibility. Results confirmed the presence of *Escherichia coli* in all the collected samples along with ampicillin and tetracycline resistant potential. This unsatisfactory hygiene status of the foods and water might develop foodborne illness and other health hazards, and thereby constitute a major food safety risk to the university students consuming meals at hall dining.

Keywords: *Foodborne diseases, University students, Pilot study, Student Health, PCR, Antibiotic resistance.*

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INTRODUCTION

Entering university is a key transitional phase for a young teenager from the secondary level to the graduate level [1]. They face several challenges to adjust in a new environment with different surroundings and a greater freedom than before [1]. Often these changes influence their eating behavior that includes irregular food intake, consuming nutrient deficient and less quantity food, skipping meals, and lack of physical activity [1, 2]. Usually, public universities of Bangladesh encourage on-campus residential facilities for students (commonly known as halls) and the hall dinings are solely responsible to meet students' dietary demands. According to the University Grant Commission of Bangladesh's annual report 2014, there are a total of 129 operational universities in the country, of which 92 are private and 37 are public universities [2]. These residential facilities (halls) provide an easy and cheap way of living for beginners especially students from distant cities and lower socio-economic backgrounds [2]. But, in many cases, their eating patterns and dietary habits faced a challenge to negotiate the new food source and its availability [3]. Previous studies indicate that improper food handling practices contributed to approximately 97% of foodborne illnesses in food service [4]. The rate of food poisoning is more than 70%

in the catering industry as compared with any other food-related sectors [5].

Foodborne diseases (FBD) resulted in considerable morbidity, mortality and economic loss all over the world and increasingly causing a wide spectrum of illnesses [6, 7]. The presence of 22 different kinds of FBD causing bacteria, protozoa and virus have been reported in 2010 [8]. Although viral contamination accounted for 50% of the total foodborne related illnesses, most cases of foodborne related hospitalization and deaths have occurred by bacterial contamination [9]. FBD causes approximately 76 million illnesses, 325000 hospitalizations and 5000 deaths annually in the USA [10]; whereas 236600 cases, 21138 hospitalization and 718 deaths in the wales and England [11]. More than two million deaths have been reported in the developing countries every year due to FBD [12]. Approximately 30 million people suffer from foodborne related illnesses each year in Bangladesh [10]. Apart from that, FBD imposes a great economic burden, accounting for 50–80 billion USD annually in health care costs, lost productivity, and diminished quality of life in the USA [13, 14]. Dysentery, diarrhea, abdominal pain, fever, headache, and vomiting are the most common FBD that are the major health concerns in Bangladesh, where transmission occurs mainly through contaminated foods and water.

Conditions become more severe for the mass population with the development of antibiotic resistance [15]. World Health Organization (WHO) describes antibiotic resistance as the largest threat to public health that could affect anyone irrespective of their age and country of residence. At least 2 million people become infected with bacteria that are resistant to antibiotics and at least 23,000 people die each year as a direct result of these infections in the USA, stated by the Centers for Disease Control and Prevention (https://www.cdc.gov/drugresistance/threat-report-2013/index.html). A major impedance to public health could arise if the antibiotic resistance determinants started to transfer to other disease-causing bacteria of humans [16]. A growing number of studies have been suggested that raw food plays a major role in the act of spreading the microbial resistance gene to humans through the food chain [17].

A recent study on the hygienic status of food preparation facilities of 32 randomly selected primary and secondary schools in Mpumalanga province in South Africa identified that only 3%, 6% and 16.2% satisfactory result of the benchtop, dry storage surfaces, and food contact surfaces, respectively [18]. The literature suggests that the transitional period during adolescent is the critical phase to determine dietary habits and nutritionally highly demanding to maintain good physical and mental health [19, 20]. Considering the scarce evidence in Bangladesh, this study aims to assess the hygienic conditions, bacterial contamination and antibiotic resistance pattern in the supplied foods and water of four residence halls in a university.

MATERIALS AND METHODS

Study area

Shahjalal University of Science and Technology (http://www.sust.edu/), is one of the highly reputed public universities in Bangladesh, located in the periphery of the Sylhet metropolitan city (Kumargaon), Bangladesh selected as the study area. The university has been established in 1987 in a total area of 320 acres [2]. It has five student dormitories on the campus; among them, three are allocated for boys and two for girls. Each of the dormitories has a dining facility to supply breakfast, lunch, and dinner. Among the five main student halls, four halls were taken under this study, that include 1st girl's hall (Shahid Jononi Jahanara Imam Hall), 2nd girl's hall (Begum Sirajunnesa Chowdhury Hall), 1st boy's hall (Shah Poran Hall), and 2nd boy's hall (Bangabandhu Sheikh Mujibur Rahman Hall) (Fig. S1).

Study population

Students (100) from the second year to the fourth year from different disciplines were recruited as the study subject to assure enough diversity. First-year students were excluded due to their limited exposure to the foods of hall dining (Table 1).

Table 1. Characteristics of the participants

Total number of students	N=100
Gender (% of female)	50%
Age (yrs)	18-22
Study year	BSc (2nd to 4th year)
Residency (% living in the student dormitory/hall)	73%

Study design and ethical issues

Ethical approval has been taken from the university ethics committee prior to data collection. The questionnaire-based survey was conducted from September 2016 to December 2016. According to the previously recommended methodology [21], a semi-structured question guide (Table 2) was developed by the research team. After getting the initial verbal consent, the survey questionnaire was provided to students. The participant rated all the questions in four different categories such as very satisfied, satisfied, dissatisfied, or very dissatisfied according to their personal opinion.

Table 2. Focus group question guide

Question type	No	Question
Introductory	1	What is the academic year of the participant?
	2	Which subject is the participant studying?
	3	What is the gender of the participant?
	4	Where is the residence of the participant?
Main	5	How satisfied is the participant on various parameters related to hall dining?
	6	Did the participant feel any sickness ever after taking the food from hall dining?
	7	What are the putative disease-causing food items available in the hall canteen?
Ending	8	Do you have any comments and suggestions about the dining hall?

Data analysis

After collecting the survey data, a simple database was created by using Microsoft Excel 2010 and the opinions of the students were converted as scores like 2, 4, 6, and 8 for their level of very dissatisfied, dissatisfied, satisfied, and very satisfied categories, respectively (Fig. 1A). The mean score was represented to analyze the level of satisfaction.

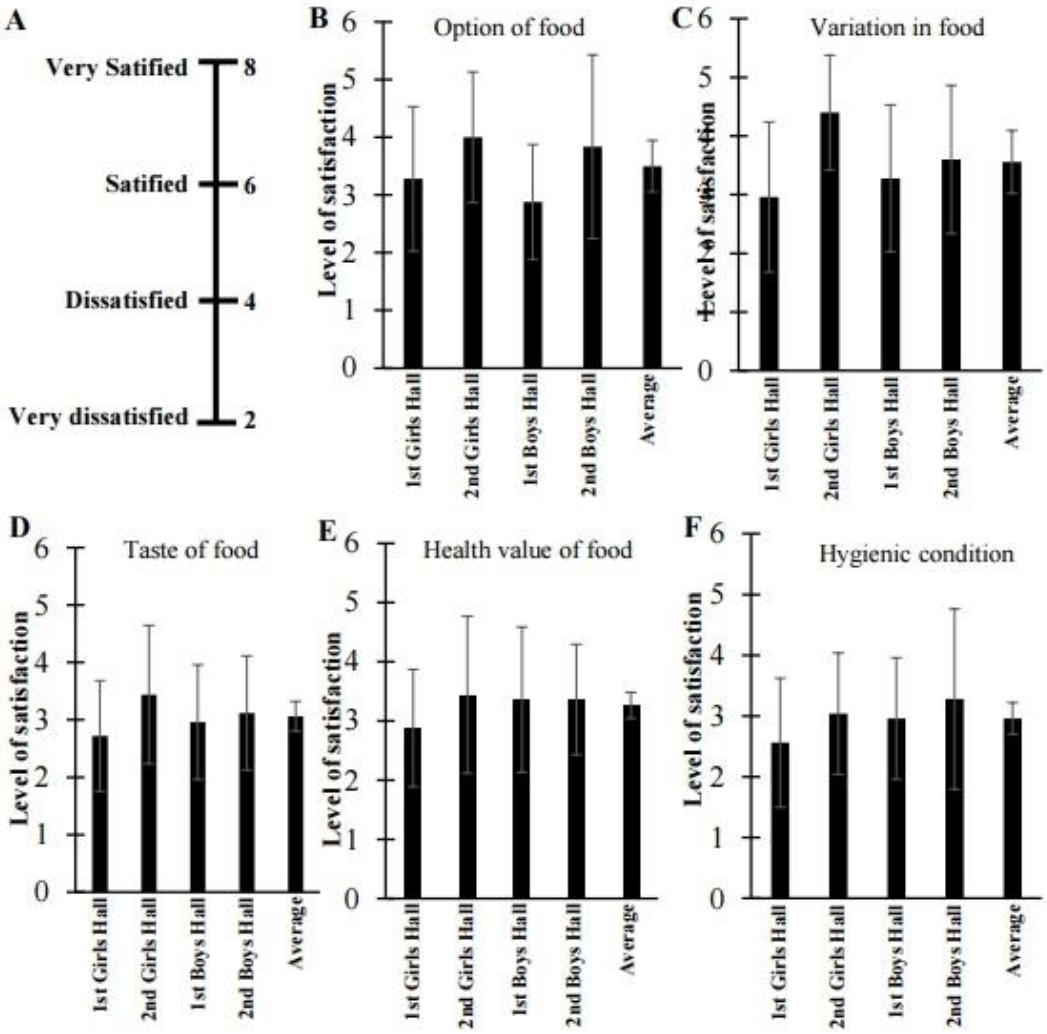


Figure 1. The level of student satisfaction in different aspects of hall dining food. (A) The level of satisfaction was measured on the scale of 2 to 8 where 2, 4, 6, and 8 indicates very dissatisfied, dissatisfied, satisfied, and very satisfied; respectively. The level of student's satisfactory level was analyzed against the availability of hall dining food options (B), variation in food (C), quality and taste of the offered foods (D), health value (E) and hygienic condition (F). In all cases (B-F), data were presented as bar diagram with standard deviation for each of four different hall dining and an average of all.

Bacteriological sample preparation and DNA extraction

Two samples (Drinking water and cooked pulse) were collected from each of these four residential halls in an autoclaved conical flask and incubated in Luria broth (LB) media at 37ºC with a rotation of 180 rpm (Fig. S2 and S3). DNA was extracted from the bacteria by boiling at 65ºC for 10 minutes in 1xTBE containing Triton X-100 [22].

PCR design and Amplification condition

PCR was carried out by using a thermal cycler (little genius, BIOER Technology Co. Ltd.) with appropriate primers (Table 3) ordered from Macrogen (https://dna.macrogen.com/eng/). A 25µl PCR mix contains 5 µl 5x Green *Flexi* buffer, 2 µl of 25 mM $MgCl_2$, 1µl of 10 mM dNTPs, 1µl of 10 mM each primer, 0.2 µl of 5 U/µL Dream Taq DNA Polymerase (Thermo Scientific, USA), 13.8 µl nuclease-free water, and 1µl of template DNA. The amplified PCR products were separated in a 1.5% agarose gel and visualized after ethidium bromide staining.

Table 3. Primers used in the study

Organism	Target gene	**Primer sequences (5' to 3')**	**Product size, bp**	**References**
E. coli	*afa*	FOR- GCT GGG CAG CAA ACT GAT AAC TCT C REV- CAT CAA GCT GTT TGT TCG TCC GCC G	750	[25]
S. typhimurium	*fimA*	FOR- CCTTTCTCCATCGTCCTGAA REV- TGGTGTTATGCCTGACCA	85	[26]

Antibiotic Resistance Test

Antibiotic Resistance tests were carried out by the disk diffusion agar method [23]. Small disks with a standard amount of antibiotics including ampicillin, tetracycline, streptomycin, and ciprofloxacin were placed onto an agar plate containing evenly spreader cultured cells. The Petri dishes were incubated at 37ºC for overnight, and the clear inhibition zone of bacterial growth was measured with a ruler in millimeter (mm) and compared with that of standard [24].

RESULTS

Characteristics of the subjects

A questionnaire-based survey of 50 male and 50 female students from four different halls was carried out. The same number of participants (25) was taken from each of the four student halls. The ratio of male and female students was 1:1 with an age range of 18-22 years. The majority of students (73%) were resided inside the hall and taken foods regularly in the hall dining. Factors influencing the quality of food, level of satisfaction, eating behavior and health status of university students were analyzed.

Analysis of the food status of hall dining

1. Student satisfaction regarding the options of hall dining food menu

First of all, the students were asked about the availability and options of hall dining foods. Most of the dining has very limited food options. The average scores of satisfaction level with hall dining option for the 1st girl's hall, 2nd girl's hall, 1st boy's hall, and 2nd boy's hall was 3.28, 4.00, 2.88 and 3.84; respectively with an overall average of 3.50 (Fig. 1B). This result indicates that students are dissatisfied with the existing food facilities of hall dining.

2. Variation of food items in hall dining

The average score of satisfaction level regarding the food variation for the 1st girl's hall, 2nd girl's hall, 1st boy's hall and 2nd boy's hall was 2.96, 4.40, 3.28 and 3.60; respectively with an average of 3.56 (Fig. 1C). This result suggests the dissatisfaction of the students regarding food variation of hall dining.

3. Quality and taste of the offered foods

Taste is an important factor that influences food choice and appetite. We have asked them regarding the quality of the offered food observing by the naked eye and taste by eating. The result was even worse than the previous two parameters. The average scores of satisfaction level with the quality and taste of the offered food for the 1st girl's hall, 2nd girl's hall, 1st boy's hall, and 2nd boy's hall was 2.72, 3.44, 2.96 and 3.12; respectively with an average of 3.06 (Fig. 1D).

4. The health value of the offered foods

Most of the students have minimum knowledge about the nutrient requirement and their available food source. We have asked them regarding their opinion about the health status of the food in terms of nutrition and a balanced diet. The average scores of satisfaction level with the nutritional status of the offered food for the 1st girl's hall, 2nd girl's hall, 1st boy's hall, and 2nd boy's hall was 2.88, 3.44, 3.36 and 3.36; respectively with an average of 3.26 (Fig. 1E).

5. Hygienic condition of offered foods and dining environment

Students are aware of their good health to a certain extent. Most of the graduate students have a minimum level of hygienic precaution regarding food

processing, handling, and serving. When they were asked about it, a minimum level of satisfaction from all sources was observed. The average scores of satisfaction level with the overall hygienic status for the 1st girl's hall, 2nd girl's hall, 1st boy's hall, and 2nd boy's hall was 2.56, 3.04, 2.96 and 3.28; respectively with an average of 2.96 (Fig. 1F).

Health status of the students

The quality and quantity of intake food are directly linked to the physical and mental health status of any human being. We were then tried to understand the effect of these low satisfactory hall dining options on their physical health.

1. Frequency of foodborne illness

A mixed pattern of sickness frequency was observed among the four different locations and gender. Students were asked about the frequency of foodborne illness. The students from the 1st girl's hall mentioned that about 9% of students became sick frequently, 16% occasionally, and 15% never fall in sick; while the 16% didn't comment (Fig. 2A).

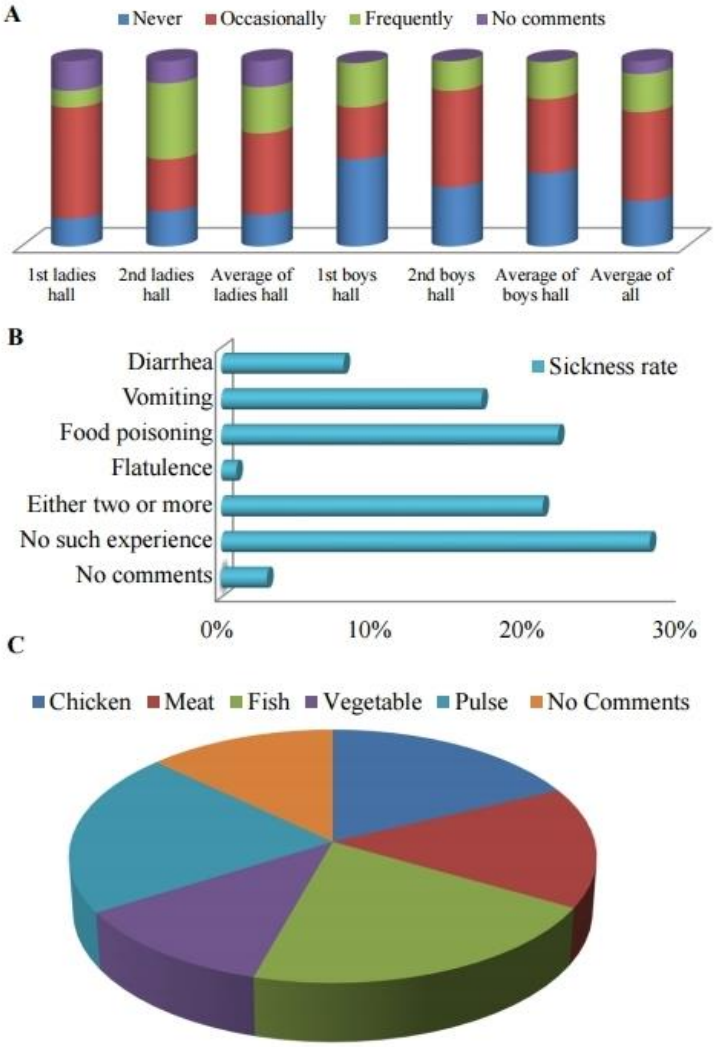


Figure 2. Effect of hall dining on health status. (A) The frequency of sickness was measured as four different categories-never, occasionally, frequently and no comments. In the bar diagram blue, red, green and purple color indicates the student's opinion as never, occasional, frequent and no comment. (B) This frequency of four different types of disease such as diarrhea, vomiting, food poison, and flatulence was measured based on their opinions. A significant number of 21% of students experienced either two or more of these diseases, while 28% of students didn't experience any of them recently. (C) The majority of the students suspect chicken and fish as contaminated food that causes this kind of disease.

Surprisingly, the rate of frequent sickness increases up to 41% in the 2nd girl's hall in spite of better satisfaction to hall dining as compared with the 1st girl's hall (Fig. 1). Participants from the boy's hall expressed 20% of frequent sickness, 40% of occasional sickness and 40% of never sickness. The rate of disease frequency was found to be highly fluctuating in respective of gender and location. As most of the students experienced frequent FBD symptoms, the students were asked about some common illnesses, such as diarrhea, flatulence, food poisoning and vomiting. A total of 28% didn't

experience any such illness in the last one year, while another 3% didn't provide any information. However, 22%, 17%, 8% and 1% of the participants had experienced symptoms of food poisoning, vomiting, diarrhea, and flatulence; respectively (Fig. 2B). Importantly, 21% of participants experienced either two or more symptoms out of four.

2. Suspected disease-causing food items

As most of the students were suffering from FBD, they were asked about the suspected food that might be contaminated or causing the frequent health problem. According to their opinion, processed fish and pulse are the most suspicious (on an average of 21%), followed by chicken, red meat, and vegetable (Fig. 2C). However, 13% of the participant did not emphasize the contribution of any food for their illnesses or symptoms

Thus, it could be inferred that the food items available in the hall dining could not able to satisfy the students as most of the parameters laid between very dissatisfactory to dissatisfactory levels satisfaction by the consumers (students). Moreover, most of the participants experienced frequent and severe FBD. Thus, the food might be contaminated with microbes due to the unhealthy and unhygienic environment of dining/employed workers. So, food served by the hall management affects the student's health severely which may cripple the youth power.

Identification of microbial contamination

A PCR based technique was used to identify and characterize the foodborne pathogens in food samples collected from the hall dinings. Genomic DNA was extracted from the cultured bacterial samples by boiling at 65ºC for 10 minutes in 1xTBE buffer. Most of the samples showed the presence of high-quality intact DNA of more than 10 kb in size, with minor degradation in a few samples (Fig. 3A).

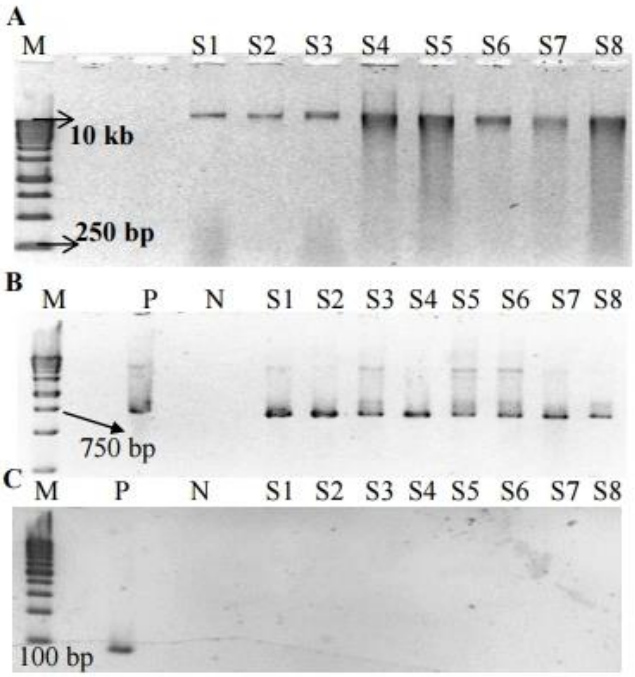


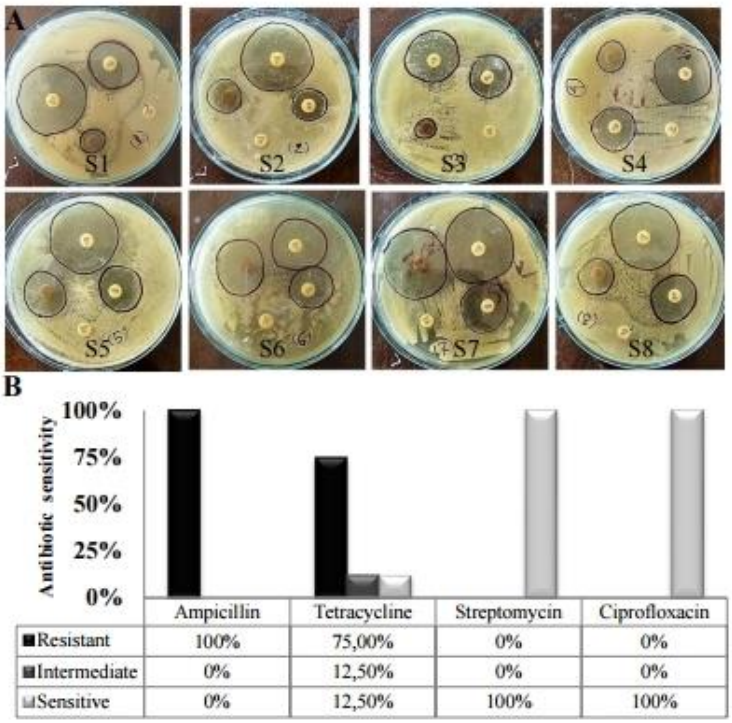
Figure 3. Molecular identification of microbial contamination. Two food samples (Cooked Pulse and Water) were collected from each of the four hall dinings and analyzed to identify the microbial contamination. (A) The Image shows the isolated DNA template with minimal degradation. Lane 1 confined (from the left side) 1kb DNA ladder, lane 4-11 confined template DNA from sample-1 (S-01) to sample-8 (S-08). (B) A PCR based technique was used to detect the presence of *Escherichia coli* using *afa* gene-specific primers. (C) Similarly, the presence of *S. typhimurium* was investigated using *fim*A gene-specific primers. Here, M= 1 kb DNA ladder, P= respective positive control, N= no template control; and S1 to S8= the collected samples from four different hall dining.

Species-specific primers were used to confirm the bacterial species by PCR. We have tested all the samples by PCR using afa [25] and fim A [26] primers to detect *E. coli* and *S. typhimurium*, respectively (Table 3). It has been inferred that all the samples were contaminated with *E. coli* (Fig. 3B), as all of them are showing the band of similar size as the positive control (750 bp). But, the samples do not have any corresponding band for *S. typhimurium* (Fig. 3C) similar to the respective positive control. The absence of a band in the samples indicates the non-existence of *S. typhimurium* while the presence of a band similar to positive control confirms the presence of *E. coli*. These PCR based analyses clearly revealed the

presence of bacterial contamination in the offered food of hall dining.

Antibiotic Susceptibility Test

All the bacterial isolates recovered from the analyzed food samples were tested for antibiotic resistance against the four most popular antibiotics. The rate of antibiotic resistance was calculated based on the previous standard inhibition zone chart [24]. The inhibition zone for each sample (Fig. 4A) against four experimented antibiotics (Fig. 4B) clearly indicated that the bacterial isolates were 100% resistant to ampicillin, and 100% sensitive to streptomycin and ciprofloxacin. However, in the case of tetracycline, a mixed pattern was observed, where 75% showed resistant, 12.5% showed sensitive, and the rest 12.5% exhibited intermediate sensitive. These results revealed that the development of progressive antibiotic resistance bacteria, which has a pernicious effect on public health.



	Ampicillin	Tetracycline	Streptomycin	Ciprofloxacin
Resistant	100%	75,00%	0%	0%
Intermediate	0%	12,50%	0%	0%
Sensitive	0%	12,50%	100%	100%

Figure 4. Antibiotic resistance in the cultured food samples. The rate of antibiotic resistance was measured based on the zone of inhibition zone against four different types of antibiotics such as ampicillin, tetracycline, streptomycin, and ciprofloxacin. (A) The inhibition zone of bacterial growth for the collected and cultured eight samples (S01 to S08) was tested for their antibiotic resistance profile. (B) Results showed 100% of the microbes present in the samples were resistant to ampicillin, but sensitive to streptomycin and ciprofloxacin; while tetracycline showed a mixed pattern.

DISCUSSION

When graduate students admitted into a university residential hall of a public university in Bangladesh, they have to go away from their family, their own ambiance, personal accessories and enter into a completely new environment in which their surroundings, lifestyle, eating habits, behavior almost everything changed. Various influences such as individual, societal, academic culture, and environmental factors largely affect the eating behavior and dietary intake of students [2]. Lack of family control, minimum budgets, social support, as well as increasing self-independence and product price are the major influencing factors of students' eating habits [16]. Moreover, they have to adapt themselves to their new circumstances including outside foods, common washrooms, sharing room and so on. Among the different problems, one of the notable issues is to ensure hygienic food and water since they face many health problems associated with foodborne illness.

This is one of the preliminary studies where we investigated the overall health status of both male and female graduate students correlating with the dining foods and water at a public university in Bangladesh. From the questionnaire-based survey data, we found that most of the students from both male and female residential halls of SUST are very dissatisfied regarding different food-related aspects of their hall dining (Fig. 1). Students were very dissatisfied with the variety of food items, quality, and taste of foods, health values and hygienic conditions of foods offered in hall dining (Fig. 1). It has been reported that changes in food preparation, handling and distribution might increase the opportunities for food contamination [27]. Moreover, students complained that hall dining workers are not aware of the hygienic

methods of food processing and handling. The result strongly revealed that the foods and water supplied in the hall dining were failed to ensure mental satisfaction as well as the sound physical health of the graduate students. As a result, students frequently experienced severe illness and consequently, the student suffers from stunted physical and mental development and never reaching full potential in their academic level.

The results strongly suggested that the foods and water supplied in the hall dining are contaminated by foodborne microbes leading to the development of FBD (Fig. 3). It has been assumed that one-third of the global total population are infected by FBD every year [28]. WHO has been stated that 31 foodborne hazards caused 600 million foodborne illnesses and 420,000 deaths in 2010. The global burden of FBD by these 31 hazards was 33 million disability-adjusted life years. Suspicious food, contributing outbreak factors, and specific causative hazard are the most crucial elements of foodborne disease outbreaks. National governments and international bodies have established extensive elaboration to control and improve food safety, to minimize the impact of FBD [29]. The presence of *Escherichia coli* in all the collected pulse and water samples was confirmed by PCR (Fig. 3B). *E. coli* is a gram-negative, rod-shaped intestinal pathotype in the *Enterobacteriaceae* family that causes diarrhea [30]. Most *E. coli* organisms are harmless but two pathogenic variants of *E. coli* such as pathovars or pathotypes has been widely studied in humans, animals, food, and environment due to their great impact on morbidity and mortality worldwide [30]. Dysentery occurs within 12 to 72 hours after ingestion of the enteroinvasive *E. coli* contaminated food. Similarly, *Salmonella typhimurium* is another gram-negative non-typhoidal *Salmonella* spp. bacterium causes typhoid-like disease in mice but not in humans [31]. The disease is also characterized by vomiting, nausea, diarrhea, abdominal cramps. Although *S. typhimurium* was absent in all the samples, the presence of *E. coli* might be directly correlated with the diarrheal experience of the students (Fig. 2B).

Surprisingly, all the bacterial isolates were found to be 100% and 75% resistant against ampicillin and tetracycline (Fig. 4) that was used in the treatment of vomiting and diarrhea [32]. The most common reasons behind the antibiotic resistance include inadequate entrance to effective antimicrobials, incomplete therapy, overusing antibiotics without any prescription, careless use and exude prescribed courses in the middle [33]. According to a 2016 Reuters investigation in the USA, antimicrobial resistance affected 2 million infections and 23,000 deaths per year [34]. Recently, a multidrug-resistant *E. coli* has been identified from human urine samples in Rajshahi area, Bangladesh [35].

Taken together, in spite of the existing limitations, the major strength of the study was to address the concurrent issues of student health, hall dining facilities, food safety, antibiotic resistance, and foodborne illness. This is an alarming issue not only for the students but also the country in terms of economic and public health perspectives.

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SUPPLEMENTARY FIGURES

Fig. S1. Map of the Study area
Fig. S2. Collected samples.
Fig. S3. Bacterial culture in the LB media after incubation

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