

ORIGINAL ARTICLE

Microbial risk assessment of ready-to-eat mixed vegetable salads from different restaurants of Bangladesh Agricultural University campus

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ABSTRACT

Objective: The study was aimed to analyze the microbiological quality of mixed vegetable salads and to understand the risk related with its consumption from different restaurants around Bangladesh Agricultural University campus in Mymensingh.

Materials and Methods: Sixty (60) samples of mixed vegetable salads were taken from twelve (12) different restaurants in five different time points from each restaurant. In parallel, restaurant workers were asked for handling practices while the consumers were interviewed about their salad consumption pattern and whether they had experienced any health-related problems. Microbial risk assessment of *Staphylococcus* spp., *Salmonella* spp., and *Escherichia coli* was estimated by Monte Carlo simulation (10,000 iterations), an exponential model.

Results: Aerobic plate count was ranged from 7.73 ± 0.61 to 9.04 ± 0.26 log cfu/gm, *Staphylococcus* spp. from 4.64 ± 0.61 to 6.42 ± 0.53 log cfu/gm, *Salmonella* spp. from 4.75 ± 0.08 to 5.27 ± 0.53 log cfu/gm, and *E. coli* from 4.98 ± 0.20 to 6.66 ± 0.80 log cfu/gm. From the survey, it was found that total consumers had 18% chances where the male had 13% and the female had 30% chances of being infected with salads. Again frequent, average, and occasional consumers had 31%, 13%, and 0% chances, respectively, of being infected with those salads. From the Monte Carlo simulation, the calculated mean annual risks of *Staphylococcus* spp., *Salmonella* spp., and *E. coli* infection for the three exposure scenarios were found to be about 100%.

Conclusion: The study actually revealed the potential microbial contamination in mixed vegetable salads which may impact on food safety and human health. So, the findings suggest that following hygienic measures during processing and handling the microbiological quality of mixed vegetables salads can be improved.

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Introduction

Food habit in Bangladesh has been remarkably changed in recent years. Nowadays, the tendency of eating out is increasing although the food typically handled, arranged, and sold at roadside eateries and other open sites are generally unhygienic. Factors encouraging the people to eat outside of the home may be the participation of female in the job sector; lifestyle changing tendency, overtime working, away from home while voyaging, fast urbanization, and crave for advanced education and research [1].

Vegetables salad plays an essential part in our food nutrition. Salad can be defined as food made mostly of a variety of raw vegetables and fruits. Common vegetables utilize as salad consists of cucumber, onions, tomatoes, lettuce, carrots, spring onions, green pepper, radish, and other ingredients which include olives [2]. Nowadays, salads have become much popular among the people because of health-related benefits. Major part of salads consists of raw vegetables. They offer a great variety of fiber contents, vitamins, minerals, and other phytochemicals that

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functions like a good supply of antioxidants as well as phytonutrients for the enhancement of human health [3].

According to Kadariya et al. [4], salad vegetables like cucumber, coriander leaf, tomato, and carrots are contaminated with *Aeromonas* spp., *Bacillus cereus*, *Campylobacter*, *Escherichia coli*, *Salmonella* spp., *Shigella* spp., and *Staphylococcus* spp. Food-borne diseases are increasing day by day because of having raw or slightly cooked vegetables and most of the human enteric pathogens are transmitted through it [5].

Food safety issues have been given a lot of interest recently due to increasing food-related illnesses [6,7]. World Health Organization (WHO) [8] addressed food-borne illness as infectious or hazardous ailments occurred by pathogens that enter into human while ingestion of contaminated food. According to Abdussalam [9], food safety measures must be applied in every stage of the food supply system starting from raw materials collection, production, processing, packaging, storage, marketing and distribution, and preparation to ensure safe, sound, and wholesome food.

Risk analysis is a tool that has been developed for safe food production by reducing food-related illnesses [10]. Both the regulatory authorities and food processors use this tool for controlling microbial hazards to ensure microbiologically safe and sound foods for consumers [11]. Among the major elements of risk analysis, the risk assessment employs both scientific and statistical information for estimating the likelihood as well as the severity of illness or death [11,12]. For the identification of the microbial risks linked with the utilization of specific food, Quantitative microbial risk assessment (QMRA) is very helpful that also provides the measures of the level of infection caused by a pathogen in a particular population consumed a specific food [13]. This ensures that the resources are purposefully coordinated to help in limiting the risk brought about by the foodborne microorganisms.

Bangladesh Agricultural University (BAU) is a densely populated area in Mymensingh and is surrounded by a number of food vendors and restaurants. It is well known that most food vendors in and around the BAU campus use the same tools for meat and vegetables processing and bare hands for serving vegetables salad and other foods. So it is essential to determine the restaurants workers contribution to the microbial load of ready to eat (RTE) vegetables salads in and around restaurants of the BAU campus. *Staphylococcus* spp., *E. coli*, and *Salmonella* spp. are very common and great indicators of unhygienic food handling, temperature misuse, and cross contamination [14]. Thus, the present research was designed to determine the presence of pathogenic bacteria with special emphasis on *Staphylococcus* spp., *Salmonella* spp., and *E. coli* in RTE vegetables salad and risk of consuming RTE vegetables salad by predictive model (exponential) using Monte Carlo simulation.

Materials and Methods

Sampling area

The study was conducted in and around BAU campus located in Mymensingh city of Bangladesh. Fifty restaurants distributed in seven places were identified in and around the BAU campus. Among these places, four were selected on the basis of a higher number of restaurants as well as consumers' preference. The sample collection places within the study area were Kamal-Ranjit market (from two restaurants), Jabbarer mor (from six restaurants), Fossiler mor (from two restaurants), and Shesh mor (from two restaurants). The sampling program was comprised with the following segments (i) microbiological sampling of RTE vegetables salads that was ready to serve at these restaurants, (ii) a questionnaire-based study regarding salad handling practices, and (iii) verbal questioning of consumers to evaluate the consumption pattern of salads.

Microbiological samples

A total of 60 RTE vegetables salads were collected from twelve (12) randomly selected restaurants on five different time points. In all cases, each sample was placed in a labeled sterile polyethylene bag and transported to the laboratory in the icebox. Then, 15 gm of the sample was ground aseptically and mixed with 135 ml phosphate-buffered saline and ten-fold serial dilution was made followed by spread onto nutrient agar, mannitol-salt agar, salmonella-shigella agar, and MacConkey agar to determine total number of bacteria, *Staphylococcus* spp., *Salmonella* spp., and *E. coli* per gm of samples, respectively. Identification of the bacterial species was further performed by cultural and morphological examinations [15].

Assessment of salad handling practices

General observations were gotten from different restaurant workers through observational questions to assess the salad handling practices during serving. During sampling of salad, following observations were made: (i) Types of salad served in that restaurant; (ii) The place where salads are stored during serving; (iii) How the salads are kept in the eateries, (iv) Types of utensil used to serve food; and (v) Practice of the food handlers during serving of salads. Along with these, the vendors were asked about whether they received any training regarding hygienic food preparation and handling.

Verbal assessment of salads consumption patterns

For assessing consumption patterns of consumers, a total of 200 questionnaires were handed out to 100 male and 100 female consumers. They were asked to answer the following questions: (i) How old they were; (ii) From which

source they frequently consume the salads; and (iii) How frequently they consume salads from the sources. The questionnaires were collected instantly after they filled the questionnaire themselves.

Microbial risk assessment

Microbial risk assessment of food involves four steps consisting of hazard identification, hazard characterization, exposure assessment, and risk characterization [16]. In order to assess microbial risk, this study was focused on the production chain of vegetable salads and at the post salad preparation condition in various restaurants to the point of consumption following the study design described by Amoah [17].

Hazard identification

The process of microbiological risk assessment starts with hazard identification. The purpose of hazard identification was to identify the micro-organism(s) present in RTE vegetables salad samples. Three organisms as *Staphylococcus* spp., *Salmonella* spp., and *E. coli* were selected as the model QRMA organisms as they are frequently found in salads samples.

Hazard characterization (dose-response assessment)

Hazard characterization defines the adverse health effect related to the consumption of microorganisms. For dose-response assessment, an exponential dose-response model [18] was used to predict the probability of *Salmonella* spp., *Staphylococcus* spp., and *E. coli* infection on the basis of laboratory output of selected microorganisms. Mathematically, the equation used for determining probability is illustrated as,

$$P(d) = 1 - e^{-rd}$$

where $P(d)$ indicates the probability of infection, d denotes the dose (cfu) of microorganisms consumed per person per day, and r refers to dimensionless infectivity constant. The model parameter “ r ” used was 7.64×10^{-8} for *Staphylococcus* spp. [18], 3.97×10^{-6} for *Salmonella* spp. [19], and 2.18×10^{-4} for *E. coli* [20].

The final dose (d) of microorganisms consumed by per person per serving was as the product of the weight of salad consumed and the quantity of organisms present per gram of salad. For vegetables salad, the amount was used about 15 g, according to the research by Seidu et al. [21]. The final doses of *Staphylococcus* spp., *Salmonella* spp., and *E. coli* consumed per serving were inputted into the mathematical model to obtain the probability of infection per day.

Exposure assessment

This step deals with the amount of salads consumed and the frequency of consumption of salads for a specified

period of time, to assess the consumer’s exposure to microorganisms with salads for a defined time period. On the basis of consumption frequency, three exposure scenarios were assessed as frequent (daily), average (weekly), and occasional (monthly) consumption of RTE vegetables salad by consumers, as described by Amoah [17]. In all three scenarios, it assumed that pathogen levels were not reduced aside a potential growth from post salad production to the point of utilization because the items were served as raw materials and were put at room temperature for quite a while during sales. Subsequently, any expansion of pathogens numbers during sales will be transferred to the consumer.

Risk characterization

This step links the previous three steps and estimates the probability of infection resulting from exposure to hazards in every exposure scenario. The annual probability of infection for each situation was estimated from the probability of infections calculated from number of days of consumed within the year.

$$P_{\text{ann}} = 1 - [1 - P(d)]^n$$

where n denotes the number of days of exposure within the year.

Statistical analysis

All the data were incorporated in Statistical Package for the Social Sciences 25.0 (IBM Corporation, USA) for performing descriptive analysis. Experimental data were analyzed by one-way analysis of variance, appropriate and significant differences among different restaurants were made at 95% confidence level ($p < 0.05$) by Tukey’s multiple comparison test [22]. For each exposure scenario, Monte Carlo simulation was run using Microsoft excel@risk software version 7.5.0 (Palisade Corporation) sampling 10,000 iterations to measure the annual risk of infection.

Results

Microbial load

Table 1 represents the average microbial load of RTE vegetables salads. The aerobic plate count (APC), *Staphylococcus* spp., *Salmonella* spp., and *E. coli* counts, however, differed among the risk sources. Mean APC, *Staphylococcus* spp., *Salmonella* spp., and *E. coli* counts ranged from 7.73 ± 0.61 to 9.04 ± 0.26 log cfu/gm; 4.64 ± 0.61 to 6.42 ± 0.53 log cfu/gm; 4.75 ± 0.08 to 5.27 ± 0.53 log cfu/gm, and 4.98 ± 0.20 to 6.66 ± 0.80 log cfu/gm, respectively. The highest mean APC 9.04 ± 0.26 log cfu/gm was recorded for restaurant Bhai Bhai Restaurants (BBR) and the lowest value 7.73 ± 0.61 log cfu/gm for Hriday Restaurant (HDR). The

highest *Staphylococcus* spp. count 6.42 ± 0.53 log cfu/gm and lowest value 4.64 ± 0.61 log cfu/gm were recorded for Madina Restaurant (MDR) and Milon Restaurant (MLR), respectively. The highest and lowest *Salmonella* spp. count was recorded as 5.27 ± 0.53 log cfu/gm and 4.75 ± 0.08 log cfu/gm in BBR and Tripty Restaurant (TRR), respectively. The highest *E. coli* count $6.66 \pm$ log cfu/gm and the lowest value 4.98 ± 0.20 log cfu/gm were recorded for BBR and Apu Restaurant (APR), respectively. The differences in the mean APC, *Staphylococcus* spp., and *E. coli* counts recorded for RTE vegetable salads were statistically significant (p -value < 0.05).

Salad handling practices and consumption details

Here, 12 restaurants were selected in and around BAU campus, Mymensingh to observe salad preparation and handling practices. RTE vegetable salads consumption details and handling practices adopted by restaurants operators were estimated by questionnaire are given in Table 2. The results reveal that mixed salad was prepared by all of the restaurants. Among the restaurants, 100% of them kept or stored the salad at room temperature whether 83.33% were served in uncovered condition. Shared utensils were used by all restaurants to serve the salad. Restaurants workers did not use any gloves during serving to consumers. Results revealed that they did not receive any training on hygienic food preparation and handling.

The detail results of RTE vegetable salads consumption as presented in Table 3 indicate that 43% of the

respondents consumed salads from home, 9% from canteens, 44% from restaurants, and 4% from other sources. With respect to the frequency of vegetable salad consumption, 13.46% of the total respondents consumed salad once a day, 50% once in a week, and 16% once a month, while 34% at unspecified occasions from all the sources. From restaurants, vegetable salads alone were consumed every day by 36.36% of the respondents, 52.28% once per week, and 11.36% once per month.

According to survey on different types of consumers, different results had been found and listed in Table 4. There were total of 88 consumers who consumed RTE vegetables salad from different restaurants in and around the BAU campus and among them 18 consumers were found who became infected by eating RTE vegetables salad from test restaurants, where males had 13% chances and females had 30% chances of being infected. According to the assessment focused on the frequency of consumption shows that the frequent consumers were at the highest risk (13%) of being infected. People aged between 25 and 30 were recorded as at risk of 27% which was the highest among total 18% infected consumers.

Risk assessment

Staphylococcus spp.

The dose-response assessment after Monte Carlo simulation indicates the mean probability of *Staphylococcus* spp. infection was 70% (90% CI: 4%–210%) with minimum and

Table 1. Microbial load of RTE vegetables salad samples.

| Code of Restaurants | Microbial load (log cfu/gm \pm Standard deviation) | | | |
|---------------------|--|----------------------------|------------------------|-----------------|
| | APC | <i>Staphylococcus</i> spp. | <i>Salmonella</i> spp. | <i>E. coli</i> |
| TRR | 8.35 \pm 0.39 | 6.22 \pm 0.48 | 4.75 \pm 0.08 | 5.53 \pm 0.38 |
| BBR | 9.04 \pm 0.26 | 6.17 \pm 0.35 | 5.27 \pm 0.53 | 6.66 \pm 0.80 |
| MDR | 8.47 \pm 0.36 | 6.42 \pm 0.53 | - | 5.98 \pm 0.42 |
| MLR | 8.30 \pm 0.54 | 4.64 \pm 0.61 | - | 5.83 \pm 0.08 |
| HDR | 7.73 \pm 0.61 | 5.66 \pm 0.37 | - | 5.80 \pm 0.12 |
| BSR | 8.45 \pm 0.47 | 5.67 \pm 0.10 | - | 5.73 \pm 0.11 |
| MNR | 7.80 \pm 0.11 | 5.90 \pm 0.20 | - | 5.75 \pm 0.08 |
| SLR | 8.60 \pm 0.34 | 5.53 \pm 0.21 | - | 5.77 \pm 0.12 |
| MMR | 8.46 \pm 0.38 | 5.69 \pm 0.58 | - | 5.76 \pm 0.25 |
| MYR | 8.47 \pm 0.35 | 5.48 \pm 0.20 | - | 5.94 \pm 0.38 |
| SFR | 8.24 \pm 0.28 | 5.89 \pm 0.69 | - | 5.91 \pm 0.20 |
| APR | 8.21 \pm 0.67 | 5.49 \pm 0.36 | - | 4.98 \pm 0.20 |

TRR = Tripty Restaurant, BBR = Bhai Bhai Restaurant, MDR = Madina Restaurant, MLR = Milon Restaurant, HDR = Hriday Restaurant, BSR = Bismillah Restaurant, MNR = Minar Restaurant, SLR = Shiuly Restaurant, MMR = Mama Restaurant, MYR = Mayamoy Restaurant, SFR = Shafiq Restaurant, APR = Apu Restaurant.

Table 2. Salad handling practices details.

| Parameter | Parameter | Frequency (n = 12) | Percentages (%) |
|-------------------------------|---|--------------------|-----------------|
| Types of salad | Cucumber | 12 | 100 |
| | Tomato | 4 | 33.33 |
| | Carrot | 8 | 66.67 |
| Salad stored or kept for sale | Mixed | 12 | 100 |
| | Room temperature | 12 | 100 |
| | Refrigerator | 0 | 0 |
| Salad served | Covered | 2 | 16.67 |
| | Uncovered | 10 | 83.33 |
| Utensil used to serve salad | Designated serving utensil | 0 | 0 |
| | Shared utensil | 12 | 100 |
| Salad served by food handlers | Gloves protected hand | 0 | 0 |
| | Bare hand | 12 | 100 |
| | Any training received on hygienic food preparation and handling | 0 | 0 |
| | No | 12 | 100 |

Table 3. Salad consumption profile in details.

| Parameters (Consumers) | Distribution of respondents Percentage | | | |
|--|--|--------|---------------|------|
| | Male | Female | Total | (%) |
| Age (years) | | | | |
| < 20 | 16 | 24 | 40 | 20 |
| 20–25 | 55 | 62 | 117 | 58.5 |
| 25–30 | 29 | 14 | 43 | 21.5 |
| Gender | | | | |
| Male | 100 | - | 100 | 50 |
| Female | 100 | - | 100 | 50 |
| Source from which salad is consumed | | | | |
| Home | 18 | 68 | 86 | 43 |
| Canteen | 15 | 3 | 18 | 9 |
| Restaurant | 61 | 27 | 88 | 44 |
| Others | 5 | 3 | 8 | 4 |
| Salad consumption frequency (total respondents) | | | | |
| Once a day (frequent consumers) | 35 | 33 | 68 | 34 |
| Once a week (average consumer) | 50 | 50 | 100 | 50 |
| Once a month (occasional consumer) | 15 | 17 | 32 | 16 |
| Salad consumption frequency (restaurants) | | | N = 88 | |
| Once a day (frequent consumers) | 19 | 13 | 32 | 36.3 |
| Once a week (average consumer) | 34 | 12 | 46 | 52.2 |
| Once a month (occasional consumer) | 8 | 2 | 10 | 11.3 |

Table 4. Risk assessment of consumers of restaurants according to different parameters.

| Parameter | Consumers | No. of consumers (n) | Case findings (d) | Risk (d/n) | Percentage (%) |
|-------------------|------------|----------------------|-------------------|------------|----------------|
| Sex | Male | 61 | 8 | 0.13 | 13 |
| | Female | 27 | 8 | 0.30 | 30 |
| Type of consumers | Frequent | 32 | 10 | 0.31 | 31 |
| | Average | 46 | 06 | 0.13 | 13 |
| | Occasional | 10 | 0 | 0 | 0 |
| Age | <20 | 13 | 01 | 0.08 | 8 |
| | 20–25 | 45 | 07 | 0.15 | 15 |
| | 25–30 | 30 | 08 | 0.27 | 27 |

maximum values being 6.5×10^{-5} and 70.5×10^{-1} , respectively (Table 5). The mean annual risk of *Staphylococcus* spp. infection linked with the consumption of RTE vegetables salad from the test restaurants for frequent, average, and occasional consumers was 110% (90% CI: 100%–130%), with minimum and maximum risk being 100% and 200% (Table 5). This indicates that daily, weekly, and monthly

consumption of RTE vegetables salad from the test restaurants may result in approximately 110 out of 100 consumers being infected with *Staphylococcus* spp.

Salmonella spp.

The mean probability of *Salmonella* spp. infection was 89.99% (90% CI: 5%–270%) with lower and higher values being 1.24×10^{-6} and 85.27×10^{-1} , respectively (Table 5). This refers that the average probability of *Salmonella* spp. infection to consumers of RTE vegetables salad from the test restaurants is most likely to be 90% with a 95% probability that the probability of infection will exceed 5% but will however, not exceed 270%. The mean annual risk of *Salmonella* spp. infection for frequent, average, and occasional consumers was 110% which indicate that approximately 110 out of 100 consumers being infected with *Salmonella* spp. after consumption of RTE vegetable salads.

E. coli

The mean probability of *E. coli* infection was 100% (90% CI: 5%–300%). For all three exposure scenarios, the mean annual risk was 110% (90% CI: 100%–130%), with minimum and maximum risk being 100% and 130% (Table 5). This indicates that the consumer had 110% chance of becoming infected with *E. coli* after consuming RTE vegetables salads.

Discussion

All the samples collected and examined from the various restaurants found to be contaminated with *Staphylococcus* spp. and *E. coli* in this study. In addition, *Salmonella* spp. was also detected in some samples. Results from this study showed that the APC was 7.73–9.04 log cfu/gm. The APC recorded from all the test restaurants samples was not within the given range of UK Public Health Laboratory Services ($6 < 7$ log cfu/gm) [23]. APC for different salad ingredients and mixed salad have been investigated by some researchers. Abdullahi and Abdulkareem [24] found mean APC of 8.36 log cfu/gm for cabbage, 8.40 log cfu/gm for lettuce, and 6.04 log cfu/gm for cucumber. Itohan et al. [25] recorded APC ranging from 6.2 to 8.46 log cfu/gm for mixed salad vegetables. Amoah [17] recorded APC of 3.1–4.83 log cfu/gm from RTE mixed vegetables salads. The APC results obtained from this study appear relatively higher than most of the results by other researcher as well as above $6 < 7$ log cfu/gm [23]. This high APC may denote poor handling practice, wrong processing, or a general absence of cleanliness knowledge that indicates the restaurant operators observed in this study probably practiced poor level of hygienic measures. Restaurants operators should take necessary hygienic measures during vegetables salad preparation.

Table 5. Risk assessment of consuming RTE vegetable salads.

| Microorganisms | Mean dose of organisms per serving (CFU) | Mean probability of infection (90% CI) | Mean annual risk of infection (90% CI) | | |
|----------------------------|--|--|--|---------------------------|-------------------------------|
| | | | Frequent consumer (daily) | Average consumer (weekly) | Occasional consumer (monthly) |
| <i>Staphylococcus</i> spp. | 1.89×10^7 | 70% (4%–210%) | 110% (100%–203%) | 110% (100%–200%) | 110% (100%–203%) |
| <i>Salmonella</i> spp. | 2.4×10^6 | 90% (5%–270%) | 110% (100%–203%) | 110% (100%–206%) | 110% (100%–192%) |
| <i>E. coli</i> | 3.1×10^7 | 100% (5%–300%) | 110% (100%–203%) | 110% (100%–207%) | 110% (100%–194%) |

Staphylococcus spp. is a common pathogen usually carried by food handlers [4]. If the *Staphylococcus* spp. levels are higher than 4 log cfu/gm in freshly cut mixed vegetable salad, then it is potentially hazardous. *Staphylococcus* spp. count in this study was ranged from 4.64 to 6.42 log cfu/gm which was higher than the findings of Amoah [17] as 2.97–5.13 log cfu/gm. All the test restaurants have crossed the acceptable range of *Staphylococcus* spp. which indicates that test RTE vegetables salads are hazardous to health. FDA [26] also indicated that, without heat processing, staphylococci may exist in any as well as all foods of animal origin or those handled directly by human. Therefore, the high levels of *Staphylococcus* spp. identified in this study propose time and temperature misuse of the samples by most restaurant operators and their lack of hygienic handling practices during sales.

From the results, *Salmonella* spp. was recorded in tested RTE vegetable salad samples from two restaurants. From the results, *Salmonella* spp. from 4.75 to 5.27 log cfu/gm was recorded which is unacceptable. According to PHLS [23], the presence of *Salmonella* spp. in any amount is a high risk to consumers. In related studies in Accra and Lomé by Myhara et al. [27] and Adjrah et al. [3], respectively, did not report *Salmonella* spp. in any of the salad samples assessed, marginally different to the findings of this study. Consumers of those two restaurants are at high risk of *Salmonella* spp. infection. Other research studies also studied on salad and salad vegetables in Uganda [28] and Iran [29] reported no *Salmonella* spp. in any of the samples.

From results, *E. coli* was recorded from 4.98 to 6.66 log cfu/gm which crossed the acceptable range where the acceptable range is 2 log cfu/gm [23]. This indicates the high amount of *E. coli* was present on tested RTE vegetables salad samples. Consumers are at high risk of *E. coli* infection.

During this study, it was observed that most restaurants operators did not use covered containers and separate serving utensils for salads. Similar study by Amoah [17] showed that 77.78% and 66.67% canteen operators use covered containers and serving utensils dedicated for serving only salads, respectively, but most of the salad provided by various canteens were not pre heated or stored at room temperature during serving to consumers which is somehow in agreement with our study. This actually

proposes that most vendors should be well-intended and more dedicated about the safety issue and well-being of consumers.

This study disclosed that 43% of the respondents consume salad from home, 9% from canteens, 44% from restaurants, and 4% from other sources. Study conducted in Ghana revealed that 46.15%, 29.49%, 16.67%, 4.49%, and 3.21% of the respondents consumed salad from home, canteens, restaurants, street food joints, and other sources, respectively [17]. In between two studies, variation within the home was quite similar but differences observed in case of restaurants, canteens, and other sources which indicated that the tendency of the respondents to consume salad sold outside of the home is slightly higher in our country. On the frequency of vegetable salad consumption, 13.46% of total respondents consume at least once per day, 50% consume once per week, and 16% once per month while 34% consume at unspecified occasions from all the sources. For respondents who consume salad from different eateries, 36.36% consume once per day, 11.36% once per month, and 52.28% once per week. Overall the percentage of average consumers is more prevalent in this study area.

From this study, it was found that the mean annual risks of *Staphylococcus* spp. infection in three categories of consumers were 110% (90% CI: 100%–130%) means 11×10^{-1} for daily, weekly, and monthly consumers. The annual risk of *Staphylococcus* spp. infection for all three categories of consumers exceeded the WHO tolerable risk of 10^{-4} per person per year [30]. Annual risk of 10^{-1} denotes a risk of one infection out of 10 consumers. Thus, for frequent, average, occasional consumers, approximately 11, 11, and 11 out of 10 RTE vegetables salad consumers, respectively, have a possibility of *Staphylococcus* spp. infection. This indicates that there is 100% chance of *Staphylococcus* spp. infection to daily, weekly, and monthly consumers of vegetable salads from the test restaurants. However, all the consumers of RTE vegetables salads from the test restaurants were at high risk of *Staphylococcus* spp. infection.

As mentioned earlier, *Salmonella* spp. were detected in the RTE vegetables salad samples of 2 restaurants out of 12 restaurants studied. The mean annual risks of *Salmonella* spp. infection for frequent, average, and occasional

consumers were 110% (90% CI: 100%–130%). Annual risk of 10^{-1} proposes a risk of 1 infection in 10 consumers. Thus, for daily, weekly, and monthly consumers, approximately 11 out of 10 RTE vegetables salad consumers stand a chance of *Salmonella* spp. infection. That daily consumption of RTE vegetables salad from two test restaurants will most likely result in infection.

The mean annual risks of *E. coli* infection in three categories of consumers were 110% (90% CI: 100%–130%) means 11×10^{-1} for daily, weekly, and monthly consumers. The annual risk of *E. coli* infection for all three categories of consumers exceeded the WHO tolerable risk of 10^{-2} per person per year [30]. Annual risk of 10^{-1} suggests a risk of one infection in 10 consumers. Thus, for frequent, average, and occasional consumers, approximately 11, 11, and 11 out of 10 RTE vegetables salad consumers separately have a possibility of *E. coli* infection. This indicates that there is a 100% chance of *E. coli* infection to daily, weekly, and monthly consumers of RTE vegetables salad from the test restaurants. However, all the consumers of RTE vegetables salad from the restaurants were at high risk of *E. coli* infection.

According to the survey of this study, different types of consumers showed different results. There were a total of 88 consumers who consumed RTE vegetables salad from different restaurants in and around the BAU campus and among them 16 consumers were found who became infected by eating salads from test restaurants. It indicates that there was 18% chance of being infected by salad consumed by total consumers where males had 13% chances and females had 30% chances of being infected. This indicates that female consumers were more susceptible than male consumers. Consumers of < 20 years, 20–25 years, and 25–30 years old had 8%, 15%, and 27% chances of being infected, respectively, by eating RTE vegetable salads from those restaurants. This indicates that 25–30 years old consumers were more susceptible than other consumers.

Survey results (risk) were lower than that risk which was found after calculating the dose (cfu/gm) of salads using Monte Carlo simulation. There was a huge number of microorganism found in laboratory test which is harmful to consumer's health. According to that amount of microorganism present in RTE vegetables salad samples, there were about 100% chances of being infected but survey reports showed that consumers had 18% chances of being infected by consuming that salads. So it is clear to us that resistance has been developed against those microorganisms in consumer's body. Further research may be conducted in the campus of BAU using other agents, different foods, and other food service establishments to build up a far-reaching profile of microbial hazards as well as security of different food items.

Conclusion

From the study, almost all of the restaurants kept salad at room temperature during sales. Storage conditions of mixed vegetable salads, poor handling practices of salads are mostly responsible for over growth of microorganisms in salads which is hazardous to consumer's health. The annual risk of *Staphylococcus* spp., *Salmonella* spp., and *E. coli* infection after the analysis for the three categories of consumers (frequent, average, and occasional consumers) represents a potentially high risk of *Staphylococcus* spp., *Salmonella* spp., and *E. coli* infection from the consumption of RTE vegetables salad served in restaurants in and around the campus of Bangladesh Agricultural University. If salads are processed hygienically and stored at low temperature, then microbial growth will be inhibited and salads will be beneficial to our health.

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Conflict of interest

The authors declare that there is no conflict of interest toward the publication of this article.

Authors' contribution

MIY carried out the research. AAMS and ZFH collected the questionnaire data and wrote the initial draft of the manuscript. SMS and SM conducted the data analysis. SP contributed to the manuscript writing. SS and MAI designed and supervised research work, rewrite, and finalized the manuscript. The final version of the manuscript read and approved by all authors before submission.

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