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# DETECTION OF *Escherichia coli* AND *Salmonella* SPECIES FROM VEGETABLES SALAD IN DHAKA CITY

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## ABSTRACT

The vegetable salad is a popular and healthy food for human beings. It may be contaminated by a lot of microbes. This research work was conducted to isolate and identify the *Escherichia coli* and *Salmonella* bacteria from mixed vegetable salad in Dhaka City, Bangladesh from 2020 to 2021. A total number of 120 mixed vegetable salad samples were collected from different restaurants, food corners and street vendors. Bacteria were identified based on cultural, staining and biochemical properties. Total viable count (TVC) and total coliform count (TCC) were determined. The mean ±SD values of TVC (log10CFU/gm) were 6.07±0.69, 5.42±0.69 and 7.04±0.48 for restaurant, food corner and street vendor, respectively. The TCC (log10CFU/gm) were 6.20±0.59, 5.23±0.59 and 6.70±0.57 in restaurant, food corner and street vendor samples, respectively. The highest contaminations of *E. coli* and *Salmonella spp.* were observed in street vendor salads which were 15% and 7.5%, respectively. The antimicrobial sensitivity test showed resistance to ampicillin, amoxicillin and tetracycline, while sensitive to ceftriaxone, gentamicin and streptomycin for both types of bacteria. So, these bacteria are zoonotic and the salad from different food shops should be prepared hygienically prior to consumption.

**Keywords:** *Salmonella*, *Escherichia coli,* vegetables salad, prevalence, Dhaka city

## INTRODUCTION

Vegetables are important components of a healthy and balanced diet which provide an extraordinary dietary source of nutrients, micronutrients, vitamins, and fiber for humans (Ahmed, 2014). Health agencies such as World Health Organization (WHO), European Food Safety Authority (EFSA), Food and Agriculture Organization (FAO), and French Agency for Food Safety (AFSSA) encourage their consumption to protect against a range of illnesses such as cancers and cardiovascular diseases (Olaimat *et al.,* 2012). Vegetables consist of leaves, roots, tubers, fruits, and flowers. These plants or plant parts may be eaten raw as salad or added to some cooked foods like rice. Common vegetables utilized as salad consists of cucumber, onions, tomatoes, lettuce, carrots, spring onions, green pepper, radish, and other ingredients which include olives. Ready-to-eat salads (RTES) technology implies procurement of raw vegetables from different local markets, cutting, sorting, washing, drying, packaging in permeable plastics, and retailing in a cold chain regime. The increased consumption of RTE fresh vegetables, in particular, leafy greens, which are used in salad mixtures that are consumed as raw has increased the chance of foodborne illnesses associated with these products in different regions of the world (Jeddi *et al.*, 2014).

Foodborne illness is a major public health concern worldwide in terms of the number of persons affected and economic cost. An estimated 600 million almost one in 10 people in the world fall ill after eating contaminated food and 420 000 die every year, resulting in the loss of 33 million healthy life years (Khater *et al.*, 2013).The predominant bacterial types found on vegetables are lactic acid bacteria, *Corynebacterium, Enterobacter, Proteus, Micrococcus, Enterococcus, Pseudomonas, Salmonella, Escherichia coli, Staphylococcus* and spore-formers. They may also possess different types of molds, such as *Alternaria, Fusarium, and Aspergillus* growing on their surface (Sagoo *et al.*, 2003).

Vegetables can be contaminated by enteric pathogens if animal or human wastes and polluted water are used for fertilization and irrigation. A variety of microorganisms including pathogens can be

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introduced from the surface of vegetables during the processing of fresh vegetable salad. Many bacterial diseases such as food poisoning, anthrax, salmonellosis, listeriosis, Crohn's disease and arthritis have been reported to be caused through consumption of contaminated vegetable salad by pathogenic microorganisms. Pathogens present in contaminated foods may harbor virulence genes, toxins and enzymes, which aids in the pathogenesis of infectious diseases. Pathogens present in contaminated foods may harbour virulence genes, toxins and enzymes, which aids in the pathogenesis of infectious diseases (Froder *et al.,* 2007).

Foodborne disease outbreaks originating in prepared raw green vegetable salads were more likely to occur on commercial food service premises than outbreaks from other sources, with restaurants and hotels accounting for maximum outbreaks. In Bangladesh, food-borne enteric disease is responsible for one-third of childhood deaths each year from diarrheal diseases (Gomez *et al.*, 2013). *Staphylococcus*, *E. coli* and *Salmonella* are very common and great indicators of unhygienic food handling and cross-contamination. The authentic and relevant research findings are not available at the Dhaka city for detailed information and control strategies development. Therefore, the present study was conducted to identify the *E. coli* and *Salmonella* bacteria from mixed vegetable salad in Dhaka City along with an enumeration of bacterial contamination from salad of the different food corners. This study further investigated the pattern of antimicrobial resistance (AMR) of identified bacteria.

## MATERIALS AND METHOD

**Study area and sample size**

The samples were collected from three categories food shop like restaurants, road side food corners and street vendors from July 2020 to June 2021. These food shops were selected randomly from ten specific areas in Dhaka City such as Farmgate, Uttara, Bashundhara, Mirpur, Kalshi, Matikata, Manikdi, Kochukhet, Mohammadpur and Agargaon.

The sample for this study was mixed vegetables salad. The ingredients of the samples were tomato, cucumber and carrot. The total number of samples was 120. The longitudinal cross sectional study was followed during the sample collection. The research work was conducted at the laboratory of Department of Microbiology and Parasitology of Sher-e-Bangla Agricultural University, Dhaka 1207.

**Sample collection and preparation**

The 100 gm of samples were weighed aseptically and taken into the zipper bag. Then the samples were transferred to the laboratory for examination. The collected samples were grinded and pasted in sterilized mortar and pestle adding PBS for homogenous mixture and then filtered. Then 0.5 ml of filtered samples were taken into the steriled test tubes having 4.5 ml of 0.1% peptone solution for making the ten-fold dilution according to the protocol described earlier (Denis *et al.,* 2016).

**Cultivation in liquid and solid media**

The liquid media used in this study were Nutrient broth (NB), Peptone broth, Methyl-red and Voges-Proskauer broth (MR-VP). The solid media were Nutrient agar (NA), Blood agar (BA), MacConkey agar (MC), Salmonella-Shigella agar (SS), Eosin Methylene Blue agar (EMB), Brilliant-Green agar (BGA) and Mannitol salt agar (MSA). 100 microliters of the processed sample was inoculated into Nutrient broth and other solid media by spread plate technique described by Caponigro *et al.* (2010). The inoculated media was incubated at 37°C temperature for 24 hours and observed 6 hour interval. The data were recorded and media was stored in the normal refrigerator for further study.

**Observation of the growth properties in various media**

Cultural properties of the *Salmonella* and *E. coli* on liquid and various solid media were observed nakedly and microscopically. The data were recorded.

**TVC and TCC counts**

The TVC and TCC were counted for determination of bacterial load according to the protocol described by (Biyani *et al.,* 2018). The EMB agar and SS agar were used for *E. coli* and *Salmonella,* respectively.

**Staining properties and microscopic observation**

The staining properties of the *E. coli* and *Salmonella* were observed under microscope. The Gram's staining technique was done according to the established protocol (Caponigro *et al.*, 2010). The colonial morphology was recorded using microscope.

**Biochemical properties observation**

Some biochemical tests (like Catalase test, Indole test, Methyl Red test, Voges-Proskauer test) were conducted according to the procedure described by Cowan and Steel (1985) and data were recorded properly.

**Antibiotic sensitivity test**

The antibiotic sensitivity tests were conducted against six types of antibiotic disc (Ampicillin, Amoxicillin, Ceftriaxone, Gentamicin, Streptomycin and Tetracycline) by disc diffusion method. The sensitivity or resistant patterns were analyzed according to Clinical and Laboratory Standards Institute (CLSI, 2007) and zone diameter interpretative standards provided by Wilson *et al.,* (2007).

**Data analysis**

The recorded data were analyzed using SPSS software (version 20.0). The means value and standard deviations (mean±SD) were determined.

## RESULTS AND DISCUSSION

**TVC and TCC counts**

There was an effect of hygienic condition on the TVC and TCC. The mean ±SD values of TVC ($(\log_{10}$CFU/gm)) were 6.07±0.69, 5.42±0.69 and 7.04±0.48 for restaurant, food corner and street vendor, respectively (Fig. 1). The obtained TVC value of samples collected from the food corner was lower than the findings of Abadias *et al.,* (2008). He reported that the total bacterial population was log 10.57 cfu/gm.

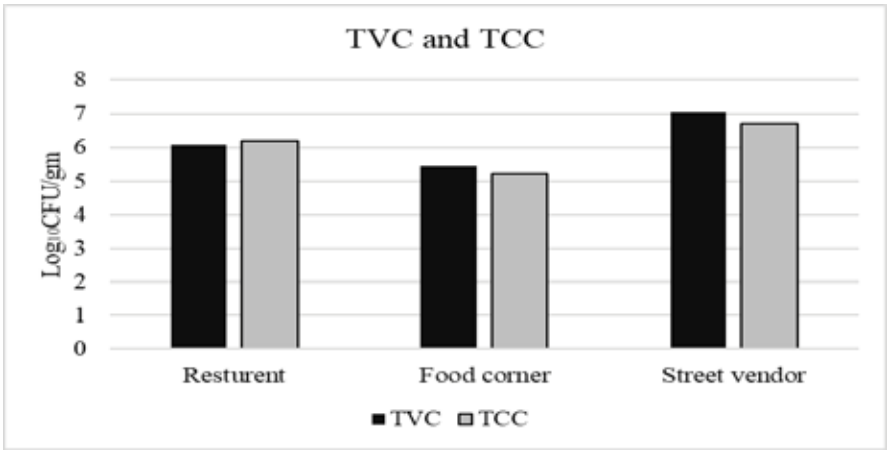


**Fig. 1. TVC and TCC of *E. coli* from sample of restaurant, Food corner and Street vendor**

In this study, the TCC ($\log_{10}$CFU/gm) for restaurant, food corner and street vendor were 6.20±0.59, 5.23±0.59 and 6.70±0.57, respectively. The observation of Khalil and Gomaa (2014) in Egypt was a wide range of TCC (log 3.63–7.17 cfu/gm) from conventional vegetable salad samples. On the other hand, Nyenje *et al.,* (2012) observed a narrow range of high TCC from log 6.94 to 8.06 cfu/gm in South Africa. This variation may be happen due to management practices in different locations, item of the salad, time duration from ready to eat, seasonal variation and food habit.

**Observation of the growth properties in various media**

The first step of isolation and identification of the *E coli* and *Salmonella* were conducted in the nutrient broth media that produced turbidity in nutrient broth in case of positive cases.

The cultural properties of these bacteria were observed in different solid media. The *E. coli* bacteria were inoculated in Nutrient agar, MacConkey agar and EMB agar. The colonies characteristics were smooth, circular and white colored colonies on Nutrient agar. The green metallic sheen colored colonies was recorded in EMB agar. In MacConkey agar plates, it was observed as bright pink to the red colored colonies due to fermentation of lactose by *E. coli*. The EMB agar was used to differentiate the coliform enteric bacteria from other enteric bacteria due to the production of acid. In acidic conditions, the dyes produce a dark purple complex which is usually associated with a green metallic sheen. The colony characteristics were smooth, circular, green colored. The colonies of other bacteria are not lactose fermenters appeared as translucent or pink.

The colonies of *Salmonella* spp. in SS agar media produced opaque, translucent, colorless, smooth and round with black centres as they were lactose non-fermenter. On Brilliant Green Agar, the *Salmonella* colonies appeared as pinkish white or red colored by a red halo in the medium, Differentiation was quite pronounced as lactose or sucrose fermenting organisms which were inhibited to produce yellow-green colonies with a green halo.

The microscopic examination of Gram's stained smears of *E. coli* and *Salmonella* revealed that the bacteria were Gram-negative, pink colored small rod shaped bacteria arranged in single, pairs or short chain.

**Biochemical properties observation**

The *Salmonella* and *E. coli* bacteria showed red color in MR positive test and colour was no changed in VP negative test. In case of catalase test, bubble formation was recorded in positive cases in both *Salmonella* and *E coli*. Red colored ring was formed in case of positive reaction in Indole test for *E. coli* and orange color developed in case of *Salmonella* bacteria for negative cases (Table 1). The cultural, staining and biochemical properties of these *E. coli* and *Salmonella* bacteria were similar according to the description of Ronald (2015) in his book.

**Table 1. Biochemical properties of *E. coli* and *Salmonella* species**

| Properties | *E. coli* | *Salmonella* |
|---|---|---|
| MR | Positive | Positive |
| VP | Negative | Negative |
| Catalase | Positive | Positive |
| Indole | Positive | Negative |

The *Salmonella* is a common foodborne pathogen that causes food contamination and has higher economic losses and significant threat to public health (Health Protection Agency, 2009). The highest observation of the *E. coli* and *Salmonella* contamination in the street vendor was 15% and 7.5%, respectively (Table 2). According to the WHO in 2016, effect of microbiological hazards such as *Salmonella* on food safety is a major public health concern worldwide. Weldezgina *et al.*, (2016) found 20.7% *Salmonella* contamination in the mixed vegetable sample in Ethiopia. Gomez *et al.,* (2013) and Toe *et al.,* (2018) observed 6.8% in Mexico and 2.6% in Ivory cost, respectively. Most of the street vendors do not take the needed precautions to avoid contamination of the raw salads during preparation and sale. In addition, in many developing countries, street food vending activities are not usually protected or regulated by the governments. So, adequate measures for treatment and cleaning of raw materials, environment and utensils together with hygienic practices of vendors must be implemented to ensure good quality of fresh vegetables and mixed vegetable salad (Alimi *et al.,* 2006).

The prevalence of *E. coli* was 5%, 10% and 15% for the restaurant, food corner and vendor, respectively. According to guidelines of the Health Protection Agency (2009), the vegetable salad will be categorized as satisfactory if the load is less than 20 cfu/g for *E. coli* and not detected for *Salmonella* in 25 gm of samples. The unsatisfactory level for *E coli* was more than 100 ($> 10^2$) cfu/g, categorized as unwholesomeness for human consumption. However, the vegetable salad was unsafe for *Salmonella*

as it was found in 25 gm of the samples. So, all vegetable salad samples from different places and food shop were considered unsafe for consumption based on this guideline.

**Table 2. The occurrence of *E. coli* and *Salmonella* has been observed in samples from all sources**

| Food shop | No. of sample | *E. coli* | | *Salmonella* spp. | |
|---|---|---|---|---|---|
| | | No. of positive sample | Occurrence | No. of positive sample | Occurrences |
| Restaurant | 40 | 2 | 5% | 2 | 5% |
| Food corner | 40 | 4 | 10% | 1 | 2.5% |
| Street vendor | 40 | 6 | 15% | 3 | 7.5% |
| Total count | 120 | 12 | 10% | 6 | 5% |

**Antibiotic sensitivity test**

The results of antibiotic sensitivity were presented in Fig. 2 and table 3. Drug resistance problem is a global public health issues now a days. Our study on antimicrobial resistance revealed that both isolates were susceptible to Ceftriaxone, Gentamicin and Streptomycin, whereas 100% resistance to Ampicillin, Amoxycillin and Tetracycline (Table 3).

**Table 3. Zone diameter (mm) interpretive standards for *E. coli* and *Salmonella* species**

| Antibiotics | Disk conc. (µg) | Zone diameter | | | *E. coli* | *Salmonella* |
|---|---|---|---|---|---|---|
| | | R | I | S | Average zone diameter | |
| Ampicillin | 25 | ≤13 | 14-16 | ≥17 | 8 (100%) | 8 (100%) |
| Amoxycillin | 30 | ≤13 | 14-17 | ≥18 | 7 (100%) | 7 (100%) |
| Ceftriaxone | 30 | ≤19 | 20-22 | ≥23 | 23 | 30 |
| Gentamicin | 10 | ≤12 | 13-14 | ≥15 | 17 | 20 |
| Streptomycin | 10 | ≤11 | 12-14 | ≥15 | 16 | 24 |
| Tetracycline | 30 | ≤11 | 12-14 | ≥15 | 10 (100%) | 8 (100%) |

Legend: R= Resistant, I= Intermediate, S= Sensitive.

According to Salmanov *et al.,* (2021), the *E. coli* was sensitive to Cefotaxime (99.1%), Ceftazidime, Piperacillin/Tazobactam and Gentamycin, but least susceptibility (70%) was observed for Amoxicillin. The *Salmonella* isolated from salad and were resistance against Amoxicillin (75%) in the observation of Nawas (2012).

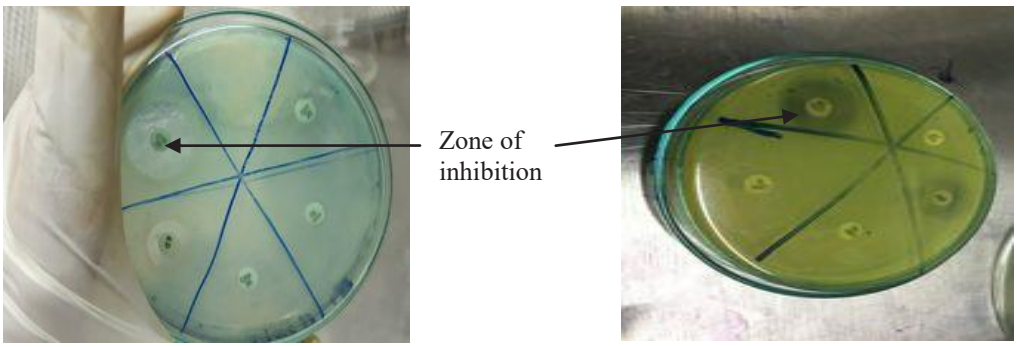


**Fig. 2. Antibiotic sensitivity test**

## CONCLUSION

The *E. coli* and *Salmonella* were identified morphologically based on their cultural properties, staining properties and biochemical properties from the vegetable salad sample. The contamination by *E. coli* was higher (15%) in street vendors and the lowest was 2.5% in food corners by *Salmonella* spp. We should ensure the wholesome condition during the salad making. Proper hygienic management of the food court should be maintained. Because these *E. coli* and *Salmonella* species are zoonotic bacteria and have different pathotypes. The Gentamicin and Streptomycin showed more sensitivity for both bacteria in cultural sensitivity test.

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