

**Microbiological Identification of isolates from salad samples and observing their antibiotic sensitivity pattern.**

**Abstract:**

Salad is often consumed fresh and thus can act as effective media for the transmission of associated pathogens. This investigation was carried out to determine the microbiological quality of salad. A total twenty-five samples were collected from twenty different area of Dhaka city. Samples were collected from chines restaurant fast food center, café, and roadside shop. According to the place of preparation samples were classified into three category such as A-(chines and fast food), B- (café ), C- (road side shop) . Samples from each category of salad were measured for total bacterial count, total coliform count and the presence of pathogenic microorganisms such as *E. coli*, *Staphylococcus aureus*, *Salmonella* spp. by standard microbiological methods. Total viable bacterial count range was from  $0.3 \times 10^3$  to  $8.3 \times 10^7$  cfu/g. The coliform and fecal coliform ranged from  $<0.4$  MPN/gm to  $>240$  MPN/gm and *Staphylococcus* spp. ranged from  $0.5 \times 10^3$  to  $1.25 \times 10^4$  cfu/g. Rates of contamination in relation to the various sources were: samples from A- category 71.4 %, B- category 87.5 % and C- category 90%. Antimicrobial susceptibility was also determined for the isolated microorganisms. Amoxicillin (10 $\mu$ g), ciprofloxacin 5 $\mu$ g), ceftazidime (30 $\mu$ g), tetracycline (30 $\mu$ g), gentamicin(10 $\mu$ g), trimethoprim-sulfamethoxazole(23.75 $\mu$ g), were used for antibiotic susceptibility testing. 100% *Salmonella* spp. was resistant to amoxicillin whereas *E. coli* was 80%. But both strains were 100% sensitive to ciprofloxacin, gentamicin, tetracycline. The results of the study demonstrate that salads available in chines restaurant, fast food center, café and road side shop are contaminated with pathogenic bacteria which are potential health threat to consumers. High standards of personal hygiene, pre-processing disinfection and proper storage temperatures will be useful to minimize the unhygienic and health risks.

**Keywords:** Salad, partially processed vegetables; Ready-to-eat (RTE); Dhaka city; Pathogen; Food borne illness

## 1. Introduction:

Different sorts of vegetables are consumed raw around the world for its profoundly wholesome value. Ordinarily salad vegetables are frequently unwashed before utilization. Vegetables are rich in carbohydrates, anti-oxidants, minerals, vitamins and fibers and frequently consumed uncooked (Slavin and Lloyd,2012, Aurin et al.,2020) The utilization rates of salad have incredibly expanded since of its therapeutic and dietary values awareness(Aycicek et al.,2006). Salad is normally consumed raw; in this manner, there's a plausibility of food infection or poisoning (Pesewu et al.,2014, Adedeji et al.,2021). Eating a lot of vegetables as portion of a low fat and high fiber diet may offer assistance decrease blood weight, chance of heart infection, stroke, diabetes, cancer and oversee weight. Alternately, raw fresh vegetables like other food items have been detailed to come in contact with an cluster of harmful microorganisms in agricultural land where industrial and domestic wastes are disposed, amid collecting, transportation and capacity and through water bodies that result within the onset of different illnesses(Noor et al.,2015).

However, salads are a common expansion to suppers and snacks in Bangladesh and as a developing nation cleanliness support is one of the major concern for the security of public health. It has been said that food and water, in specific, serve as vectors for the spread of microbial diseases, including those brought on by coliforms. There might be a few sorts of organisms present in salad samples including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni*, *Clostridium perfringens*, total aerobic and spoilage microbes, yeasts, and molds, which are concerned with genuine dangers (Łepecka et al., 2022). Among them Salmonellae, Shigellae, and enteropathogenic *Escherichia coli* are a number of vital enteric pathogens among the coliforms. In terms of human health and illness, *Escherichia coli* O157:H7 and *Salmonella* spp. are the foremost harmful food-borne bacterial pathogens. As a result, research should be done to decide the presence of pathogenic organisms in salad natural products and vegetables offered in Dhaka's conventional markets and basic supply shops, as well as the extent of this contamination (Islam,2023)

On the contrary, in the recent times, food-borne illnesses and broad resistance to antibiotic treatment could be a exceptionally genuine public health threat (Rabbi et al.,2011, Thorpe et al.,2018). In this way, the microbial vegetation of raw foods such as vegetable salads is of extraordinary concern. The washing of vegetables in chlorinated water which may decrease bacterial loads isn't common within the developing world (Adebayo-Tayo et al.,2012). Besides, the inaccessibility of consumable water for appropriate washing makes salad vegetables a significant contributor to the transmission of food borne diseases (Al Mamun et al.,2013).As salad samples are easily accessible in different regions from restaurants to as a street food so the contamination variety will be distinctive from one another. It may be due to the taking care of handle, environment and as street vendors are not exceedingly taught so lack of education can be a cause of concern. Based on overall circumstance, salad samples from distinctive categories of

restaurants were observed to identify the microbial defilement together with their sensitivity pattern against commercially accessible anti-microbials which are commonly endorsed in our nation.

## **2. Method & Materials:**

**2.1 Sample Collection & Processing:** Total 25 Salad samples from different area of Dhaka city were collected in sterilized sampling bags aseptically following the method of American public Health Association (APHA, 1998) [13] and transported under aseptic conditions to the laboratory, as soon as possible for processing on the same day of collection. Samples were classified into three categories such as A-(chines and fast food), B- (café ), C- (road side shop).

### **2.2 Enumeration of total viable bacteria**

Total viable bacteria were enumerated by spreading 0.1 µl of each sample suspension onto nutrient agar (HiMedia Laboratories Pvt. Ltd., India). After incubation at 37°C for 24 h, plates were examined (Acharjee,2023).

### **2.3 Estimation of *Escherichia coli* and *Staphylococcus aureus***

For the isolation of *E. coli*, and *S. aureus*, 0.1 ml of suspension was spread over MacConkey agar and Mannitol Salt agar (HiMedia Laboratories Pvt. Ltd., India) for each sample and incubated at 37°C for 18–24 hr. (Collee et al.,1996). The presence of *E. coli* was further confirmed by the appearance of bluish-black colonies with a green metallic sheen on the eosin-methylene blue agar (HiMedia Laboratories Pvt. Ltd., India). *S. aureus* was identified by observing yellow colonies on Mannitol Salt agar (Sultana et al.,2024).

### **2.4 Enrichment of *Salmonella* spp.**

In vitro cultivation of *Salmonella* spp. often appears difficult or produces erroneous results (false negatives) due to their viable but nonculturable properties (Sultana et al.,2024). Therefore, an enrichment technique for concentrating *Salmonella* in selenite cystine broth (SCB) was carried out. 1 ml of the homogenized sample suspension was transferred to SCB, followed by incubation at 37°C for 4 h, serially diluted to 10<sup>4</sup>, and 0.1 ml of which was spread over the Salmonella-Shigella (SS) agar (HiMedia, India) and incubated at 37°C for 24 h.(Sultana et al.,2024, Akter et al.,2021, Collee at el.,1996).

### **2.5 Biochemical tests for the confirmative identification**

All isolated bacteria were identified by standard laboratory biochemical tests according to the methods described elsewhere. The biochemical tests were the indole test, MR-VP test, catalase test, oxidase test, urease test, beta-hemolysis test, coagulase test, citrate utilization test, H<sub>2</sub>S production test, as well as mannitol fermentation test (Sultana et al.,2024).

## 2.6 Determination of antimicrobial susceptibility by disk diffusion method

Pure culture of isolates from different salad dressing samples was selected for determining antibiotic susceptibility pattern against 10 different groups of antibiotics such as azithromycin (30 µg), cefixime (5 µg), chloramphenicol (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), doxycycline (30 µg), erythromycin (15 µg), gentamycin (30 µg), meropenem (30 µg), and vancomycin (30 µg), by Kirby-Bauer disk diffusion method and as recommended by the Clinical and Laboratory Standards Institute guidelines, 2023 (Bauer et al.,1996).

## 3. Result

### 3.1 Representative isolates from the samples:

According to the study, Category C showed the highest range of bacterial samples while category A showed lowest. Coliform and fecal coliform were less in both A & B category with the log value of 2-3/gm than C category where values were ranged from 3-4/gm. On the other hand, Staphylococcal count was higher in category B & C than category A (Fig 1a). On the contrary, category A showed the highest presence of *Salmonella* spp. while the lowest one is observed in category C which is 10% only. 37% of the presence were observed in case of B category (Fig 1b). All the isolates like *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. were identified through various biochemical test and table 1 supported this statement. This present study indicates that hygiene issues are related with the microbial count of the samples.

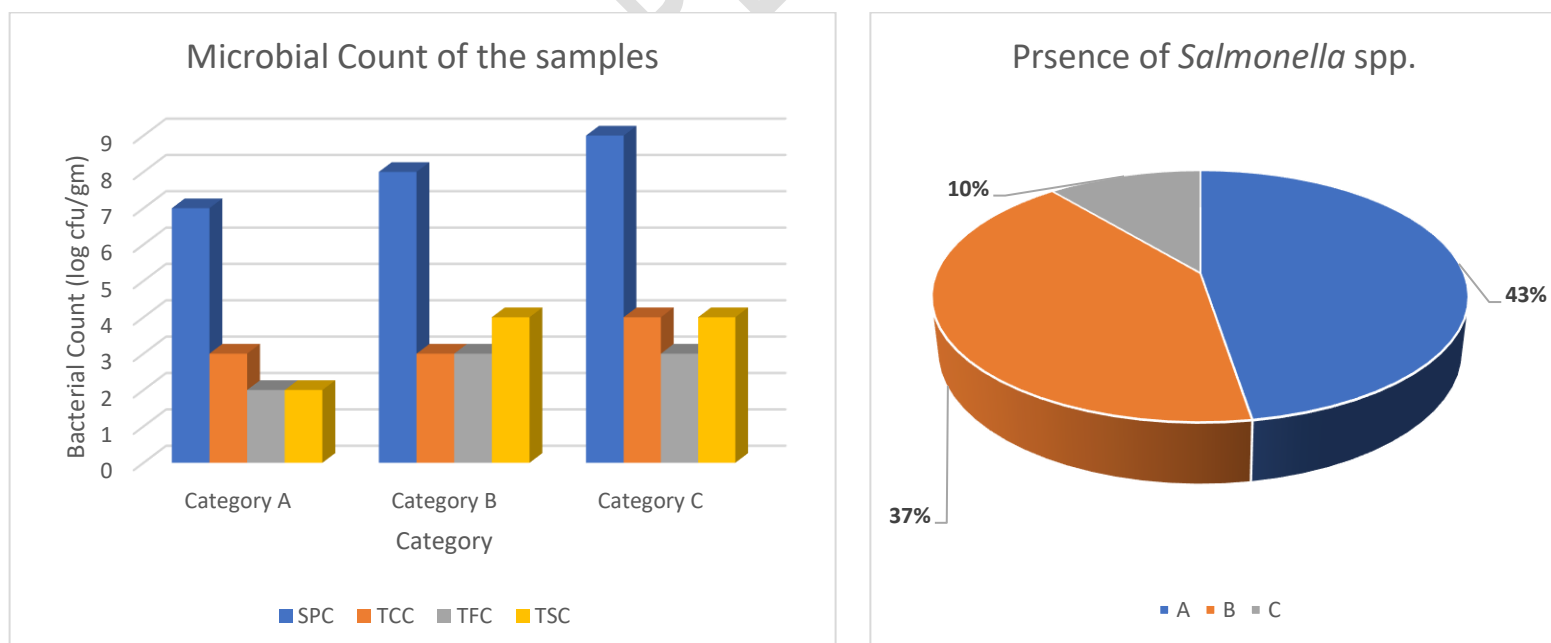


Figure 1: Microbiological survey of the samples (a) & Abundance of *Salmonella* spp. in the samples(b).

| Presumptive organism       | TSI test |        |     |                  | MR test | VP test | Catalase test | Oxidase test | Indole test | Citrate test |
|----------------------------|----------|--------|-----|------------------|---------|---------|---------------|--------------|-------------|--------------|
|                            | Slant    | Butt   | Gas | H <sub>2</sub> S |         |         |               |              |             |              |
| <i>Escherichia coli</i>    | Yellow   | Yellow | -   | -                | +       | -       | +             | -            | +           | -            |
| <i>Salmonella</i> spp.     | Red      | Yellow | -   | +                | +       | -       | +             | -            | -           | +            |
| <i>Staphylococcus</i> spp. | Yellow   | Red    | +   | +                | +       | +       | +             | -            | -           | +            |

Table-01: Biochemical properties of the identified isolates

### 3.4 Antibiotic susceptibility test of the isolates:

According to our result, it was noticeable that most of the antibiotic were sensitive against the isolates. *E. coli* and *Salmonella* spp. were 100% sensitive to ciprofloxacin, gentamicin, and tetracycline. But both strains were resistant to amoxicillin and trimethoprim-sulfamethoxazole (50% for *E. coli* & 0% for *Salmonella* spp.) showed moderate sensitivity against them. On the contrary, gentamycin (90%) and ciprofloxacin (75%) were highly sensitive to *Staphylococcus* spp. while others gave low to moderate sensitive range (30-60%) for the isolate.

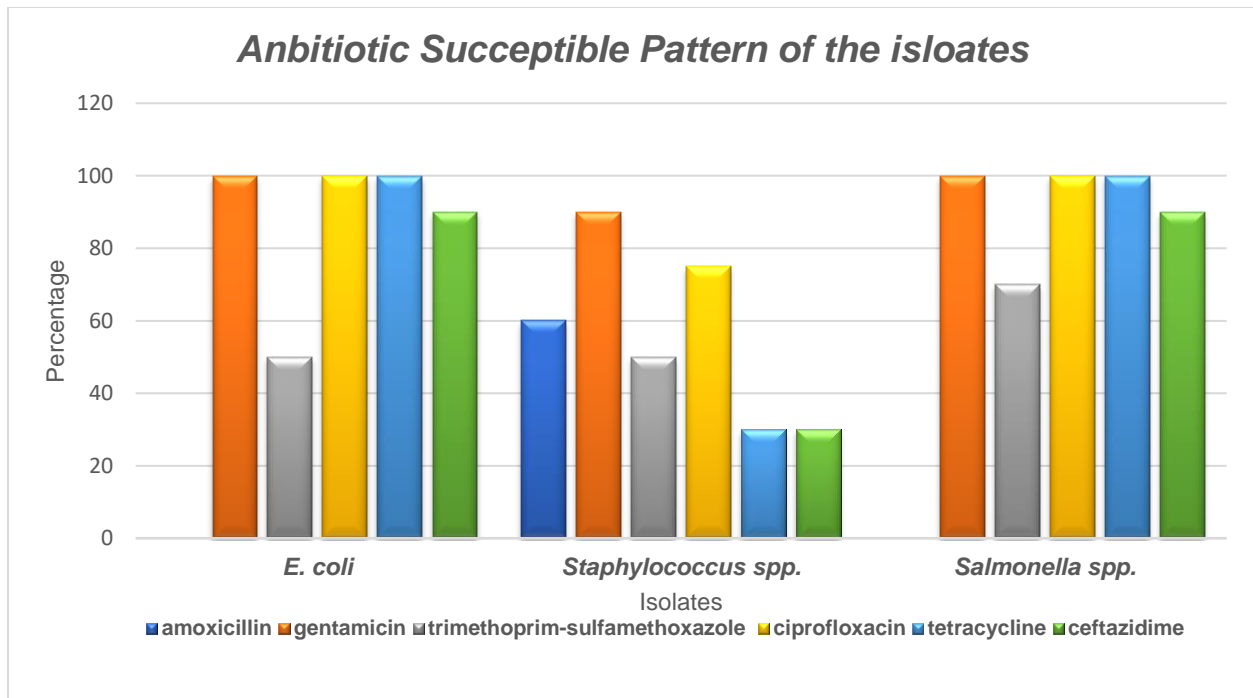


Figure 2: Antibiotic Susceptibility of the isolates

#### 4. Discussion:

The accessibility of the typical salad sample ingredients is pivotal to city inhabitants' every day lives. Foods, if handled without agreeing to sterile benchmarks, they can transmit a extend of diseases and cause a assortment of food-borne illnesses, particularly with tests. Public health is essentially affected by risky and unhygienic food handling, which leads to a range of chronic and non-chronic sicknesses. Due to a need of understanding, mindfulness, and adherence to the food rules, food contamination and food-borne illnesses are very predominant in Bangladesh. Additional bacterial contamination of the raw materials may have happened at the distributing destinations amid cutting and chopping. In this regard it was observed that raw meat and poultry as well as sauce and salad raw materials were cut and chopped using the same knife without in-between cleaning, resulting in cross-contamination between the distinctive food types (Bryan, 1988). Most of these items are generally eaten without further preparing. This sort of food can be unsafe to consumer's health if safety measures are not entirely complied with at the time of arrangement. In this manner, this current study was attempted in deciding the quality of salad available in Dhaka city. The quality and safety is decided by the nearness of indicator organisms, which are not desirable in salad and salad thing, but if they are present in tremendous number this shows the quality is exceptionally destitute and did not keep up sterile conditions and there's a extraordinary chance of presence of another pathogenic bacterium (Fröder et al., 2007).

All analyzed salad samples showed positive growth on Plate Count Agar indicating the presence of mesophilic microorganisms. The total viable bacterial counts/gm ranging from  $1.3 \times 10^3$  cfu/gm to  $8.3 \times 10^7$  cfu/gm were observed in the samples study. The maximum total bacterial count of C-category salad (Cucumber salad) collected from Gulistan and the minimum total bacterial count of the A-category salad (chicken salad) collected from Banani. As Gulistan was a very highly polluted and populated area, so salad samples were contaminated by the microbial load present in the environment. The mean value of total bacterial count of three categories ranged from 7.99 to 5.50 log<sub>10</sub> cfu/gm. Coliforms are one type of indicator microorganisms in food items. The presence of the coliform bacteria, such as *E. coli* in salad is a common indicator of fecal contamination. The total highest coliform count >240 MPN/gm were found in almost all categories of salad. But fecal coliform was lower. *E. coli* was isolated from 10 salad sample out of 25 samples among them two were A- category salad and three was B- category salad and rest from C- category salad. The presence of *E. coli* in food is undesirable because it indicates poor hygienic conditions which have led to contamination or inadequate heat treatment. *Staphylococcus aureus* is considered the third most important cause of disease in the world among the reported food-borne illness (Zhang et al., 1998). Staphylococcal food poisonings persistent cause of gastroenteritis worldwide, especially in developed countries (Vora et al., 2003). In the present study, the overall occurrence of *Staphylococcus aureus* was 19% among 30% was in C-category salad and 14.28% was B category salad samples (fig 2). In all categories of salad total staphylococcal counts ranged from 2.45 to 3.28 log cfu/gm. The overall occurrence of *Salmonella* spp. was 28 % among 12 % was in A-category salad and 12% was B category and 4% was C-category salad samples (fig 3). In this present study, all the salad samples were contaminated with the pathogens which are also observed to be present in an another study conducted in Nepal. However, the range was different and it might be due to the environmental factors, regional factors, handling process etc. (Poudel et al., 2020).

Drug resistance is a serious problem in these days that is becoming more and more risky for the global public health. Our study of antibiogram revealed that most of the isolates were susceptible towards most of the antibiotics, whereas resistance towards some antibiotics indicates that the foodborne illness can be cured through the antibiotic therapy. According to our study, *Salmonella* spp. and *E. coli* showed their 100% susceptibility to ciprofloxacin, gentamicin, and tetracycline while both strains were resistant to amoxicillin. Second highest susceptibility which was 90% was observed for both strains. In this present study, gentamicin (80%) and ciprofloxacin (75%) were the best choice to treat *Staphylococcus* spp. and Ceftazidime (30%) were almost resistant to *Staphylococcus* spp. Our result supports the another study of Nepal (Poudel et al., 2020).

This study shows destitute microbiological quality of street vended and restaurant vegetable salads sold Dhaka city, Bangladesh which are intensely contaminated with bacterial strains of public health importance delineating need of appropriate cleanliness by sellers and source of contamination. However, it was noticeable that the commonly accessible antibiotics are still valuable to treat the pathogenic

organisms found within the salad samples. But as a developing nation we ought to concentrate on the execution of hygiene maintenance as frequent use and misuses leads to the development of antibiotic resistance.

## 5. CONCLUSION:

Outbreaks of human infection associated with the consumption of raw fruit and vegetables frequently happen in developing nations and have ended up more frequent in developed countries over the past decade. Results obtained from this study showed the presence of different pathogenic bacteria on the surface of unwashed fresh raw vegetables from distinctive sorts of restaurants and street vendors in Dhaka city which demonstrates the need of legitimate cleanliness practice during and post-harvesting stage of the vegetables. Appropriate washing with water and/or pretreatment with distinctive antimicrobial agents can decrease the chance of such contaminations. On the contrary, presence of coliform microbes appeared as pointers of fecal contamination by human, animal or irrigation water and natural fertilizers. Potential pathogenic bacteria isolated were *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. which have a health risk to consumers. Our study appears that these pathogenic strains can still be killed by means of anti-microbials but as antibiotic resistance are expanding in later times so appropriate steps ought to be taken to improve the cleanliness quality of the salad and salad items. Nevertheless, the major confinement of this study was the need of molecular characterization of the isolates.

## References:

1. Slavin, J.L. and Lloyd B. (2012). Health Benefits of Fruits and Vegetables. *Advances in Nutrition*,506-516.
2. Aurin, S.A., Chowdhury, S.P., Abony, M., Rifa, J., Banik, A., Fatema, et al. (2020). Characterization of Multi-Drug Resistant Gram-Negative Bacteria Present in Fresh Leafy & Salad Vegetables in Dhaka, Bangladesh. *European Journal of Engineering and Technology Research*, 5(11),1322-7.
3. Aycicek, H., Oguz, U., Karci, K. (2006). Comparison of results of ATP bioluminescence and traditional hygiene swabbing methods for the determination of surface cleanliness at a hospital kitchen. *International journal of hygiene and environmental health*, 209, no. 2: 203-206.
4. Pesewu, G.A., Agyei, J.N., Gyimah, K.I., Olu-Taiwo, M.A., Osei-Djarbeng, S., Codjoe, F.S. et al. (2014). Bacteriological assessment of the quality of raw-mixed vegetable salads prepared and sold by street food vendors in Korle-Gonno. *Journal of Health Science*, 2(2),560-566.
5. Adedeji, A.S., Ray, S.B., Osula, G.M., Adabara, N.U., Sadiq, F.U., Adamu, B.B., Kuta, F.A. (2021). Contamination of Street Vended, Ready to Eat Vegetable Salad with Multidrug Resistant Bacteria. *Journal of Enam Medical College*, 11(3),164-72.

6. Noor, R., Malek, M., Rahman, S. *et al.* (2015). Assessment of survival of pathogenic bacteria in fresh vegetables through in vitro challenge test. *Food Contamination* 2, 15 <https://doi.org/10.1186/s40550-015-0021-3>
7. Łepecka, A., Zielińska, D., Szymański, P., Buras, I., Kolożyn-Krajewska, D. (2022). Assessment of the microbiological quality of ready-to-eat salads—are there any reasons for concern about public health? *International Journal of Environmental Research and Public Health*, 29,19(3),1582.
8. Islam, M.A. (2023). Isolation, identification, and antibiogram studies of *Escherichia coli* from salad vegetable samples sold in Dhaka city (Doctoral dissertation, Brac University),1-38.
9. Rabbi, F.A., Rabbi, F., Runun, T.A., Zaman, K., Rahman, M.M., Noor, R. (2011) Microbiological quality assessment of foods collected from different hospitals within Dhaka city. *Stamford Journal Microbiology*, 1(1), 31-36.
10. Thorpe, K.E., Joski, P., Johnston, K.J. (2018). Antibiotic-resistant Infection Treatment Costs Have Doubled Since 2002, Now Exceeding \$2 Billion Annually. *Health Affairs*,37(4): 662–669.
11. Adebayo-Tayo, B.C., Odu, N.N., Esen, C.U., Okonko, I.O. (2012). Microorganisms associated with spoilage of stored vegetables in Uyo Metropolis, Akwa Ibom State, Nigeria. *Nature and Science*, 10(3), 23-32.
12. Al Mamun, M., Rahman, S.M.M., Turin, T.C. (2013). Microbiological quality of selected street food items vended by school-based street food vendors in Dhaka, Bangladesh. *International Journal of Food Microbiology*,166(3),413-418.
13. American Public Health Association. Standard Methods for the Examination of Water and Wastewater. 20th ed. Washington DC: American Public Health Association; 1998
14. Acharjee, M. (2023). Cultural, biochemical and microscopic evaluation of some common microorganisms isolated from different environmental sources. *Journal of Advances in Microbiology Research*, 4:05-9.
15. Sultana, R., Alam, S.T., Akter, S., Mia, P., Nabila, S.S., Tasnim, S. *et al.* (2024). Microbiological Analysis of Bacterial Isolates Obtained from Salad Dressing Samples from Street Food Vendors in Bangladesh Along with their Antibiogram Profiling. *Journal of Preventive, Diagnostic and Treatment Strategies in Medicine*, 3(2):92-9.
16. Akter, T., Feroz, F., Ishma, T., Uddin, S.H., Islam, M., Saha, S., *et al.* (2021). Evaluation of microbial contamination level and the drug susceptible pattern of the isolates cultivated from famous dessert food. *Food Research (Malaysia)*.
17. Collee, J.G., Marmion, B.P., Fraser, A.J. (1996). Practical Medical Microbiology. 14th ed. New York: Churchill Livingstone, 131-49, 166-7.
18. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 1966;45:493-6.

19. Rohith, S., 2021. An investigation into the hygiene practices and food safety of street vendors outside pension pay-out points in urban poor communities in the City of Cape Town (Doctoral dissertation, Stellenbosch: Stellenbosch University).
20. Fröder, H., Martins, C.G., De Souza, K.L., Landgraf, M., Franco, B.D., Destro, M.T.(2007). Minimally processed vegetable salads: microbial quality evaluation. *Journal of food protection*, 70(5),1277-80.
21. Abebe, E., Gugsa, G. and Ahmed, M. (2020). Review on major food-borne zoonotic bacterial pathogens. *Journal of tropical medicine*,1, 4674235.
22. Vora, P., Senecal, A. and Schaffner, D.W. (2003). Survival of *Staphylococcus aureus* ATCC 13565 in intermediate moisture foods is highly variable. *Risk Analysis: An International Journal*, 23(1),229-236.
23. Poudel, R., Gautam, N., Nepal, K., Lekhak, B. and Upreti, M.K. (2020). Microbiological Quality and Antibigram Assessment of Bacterial Pathogens Isolated from Raw Salad Vegetable Samples of Kathmandu Valley. *Himalayan Journal of Science and Technology*,88-95.