Journal of Advances in Microbiology

Volume 24, Issue 12, Page 189-197, 2024; Article no.JAMB.128240
ISSN: 2456-7116

Screening the Microbiological Contamination in Salad Samples and Analysis their Antibiotic Sensitivity Pattern

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jamb/2024/v24i12883

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/128240

Original Research Article

Received: 07/10/2024
Accepted: 09/12/2024
Published: 21/12/2024

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 restaurants, fast food centers, café, and roadside shops. According to the place of preparation samples were classified into three category such as A (chines and fast

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Cite as: *Chowdhury, Umme Salma, Raquiba Sultana, and Mrityunjoy Acharjee. 2024. "Screening the Microbiological Contamination in Salad Samples and Analysis Their Antibiotic Sensitivity Pattern". Journal of Advances in Microbiology 24 (12):189-97. https://doi.org/10.9734/jamb/2024/v24i12883.*

food), B (café), C (road side shop). Samples from each category of salad were prepared to determine the total bacterial count, total coliform count and highlighted the presence of pathogenic microorganisms such as *E. coli, Staphylococcus aureus, Salmonella* spp. by standard microbiological methods. The total viable bacterial count ranged from 0.3×10^3 to 8.3×10^7 cfu/g. The coliform and fecal coliform ranged from <0.4 MPN/gm to >240 MPN/gm and *Staphylococcus* spp. ranged from 0.5×10^3 to 1.25×10^4 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 µg), ciprofloxacin 5 µg), ceftazidime (30 µg), tetracycline (30 µg), gentamicin (10 µg), trimethoprim-sulfamethoxazole (23.75 µg), 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. Maintaining 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 is a possibility 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 pressure, 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 a 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 concerns 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 *Salmonella*, *Shigella*, and enteropathogenic *Escherichia coli* are significant enteric pathogens. 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 an 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 are 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). "Furthermore, the lack of access to drinkable water for proper cleaning makes salad veggies a major source of food-borne illness transmission" (Al Mamun et al., 2013). Since salad samples are widely available in many locations, ranging from eateries to street food, the contamination types

will differ 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. METHODS AND 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) 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 up to 10^{-4}, 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_2S 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), gentamicin (30 µg), meropenem (30 µg), and vancomycin (30 µg). Mueller-Hinton agar (Difco, Detroit, MI) was equally coated with the loop-full inoculum from the stock culture. The disc-diffusion method (Kirby Bauer technique) was then used to apply the antibiotic discs to the bacterial lawn's surface. After that, the plates were turned upside down and incubated for twenty-four hours at 37°C. To confirm the drug sensitivity or resistance array, the zone of inhibition was measured after incubation and compared to the standard chart (Bauer et al.,1996).

3. RESULTS

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.

3.2 Antibiotic Susceptibility Test of the Isolates

According to our result, it was observed that most of the antibiotic were effective 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* against trimethoprim-sulfamethoxazole & 0% for *E. coli* and *Salmonella* spp. against amoxicillin) showed moderate sensitivity against them. On the contrary, gentamycin (90%) and ciprofloxacin (75%) were effective against *Staphylococcus* spp. while others gave low to moderate sensitive range (30-60%) for the isolate. The bacterial isolates have been gaining the antibiotic resistance day by day due to the lack of proper guidelines of using drugs, poor practices to consult with physician, bacterial mutation influenced by environmental factors and climate changes.

4. DISCUSSION

The accessibility of the typical salad sample ingredients is pivotal to city inhabitants' everyday lives. Foods, if handled without agreeing to sterile benchmarks, they can transmit an extend of diseases and cause an 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 are 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 an 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 1.3×10^3 cfu/gm to 8.3×10^7 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 log10 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 (Nawas et al., 2012). 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).

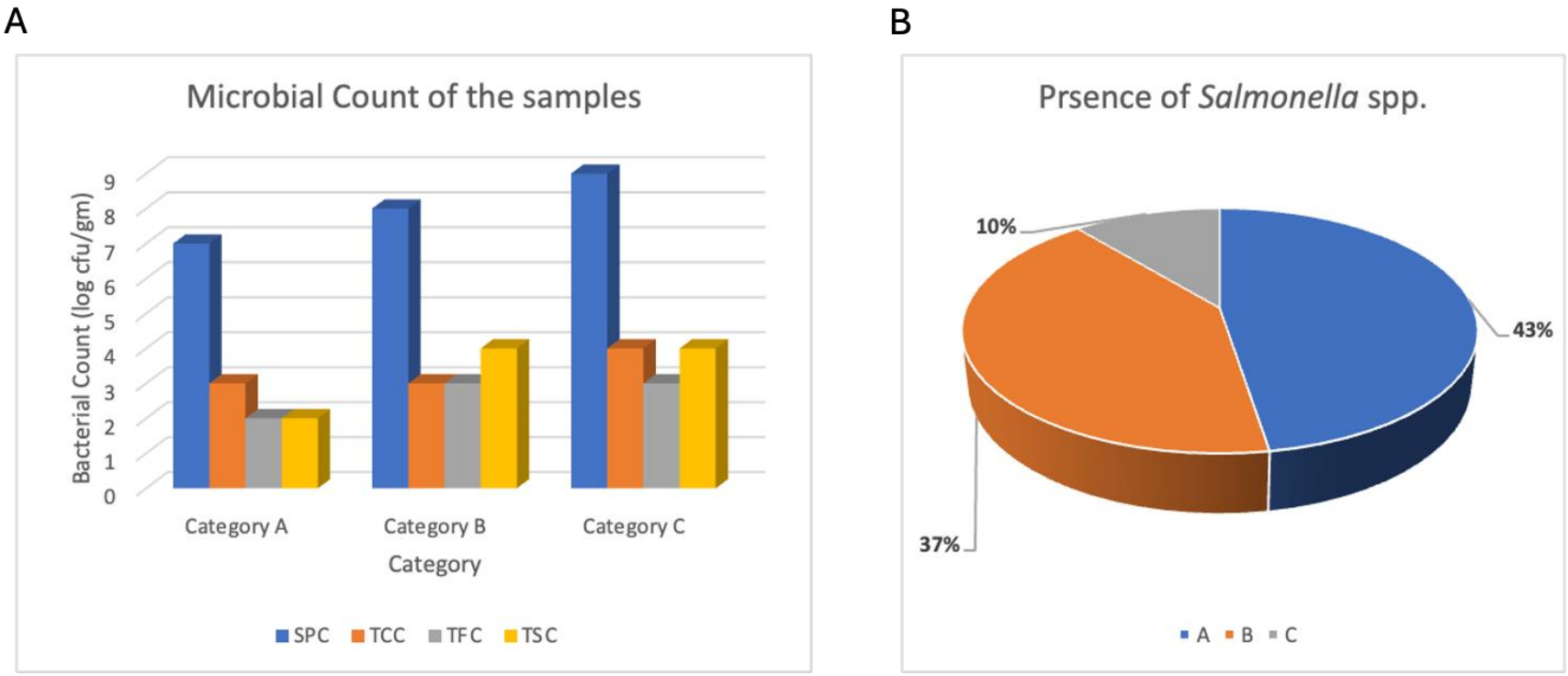

Fig. 1. Microbiological survey of the samples (A) & Presence of *Salmonella spp.* in the samples(B). In figure A: SPC: Standard Plate Count, TCC Total Coliform Count, TSC: Total Staphylococcal Count

Table 1. Biochemical properties of the identified isolates

Presumptive organism	TSI test				MR test	VP test	Catalase test	Oxidase test	Indole test	Citrate test
	Slant	Butt	Gas	H2S						
Escherichia coli	Yellow	Yellow	−	−	+	-	+	-	+	-
Salmonella spp.	Red	Yellow	−	+	+	-	+	-	-	+
Staphylococcus spp.	Yellow	Red	+	+	+	+	+	-	-	+

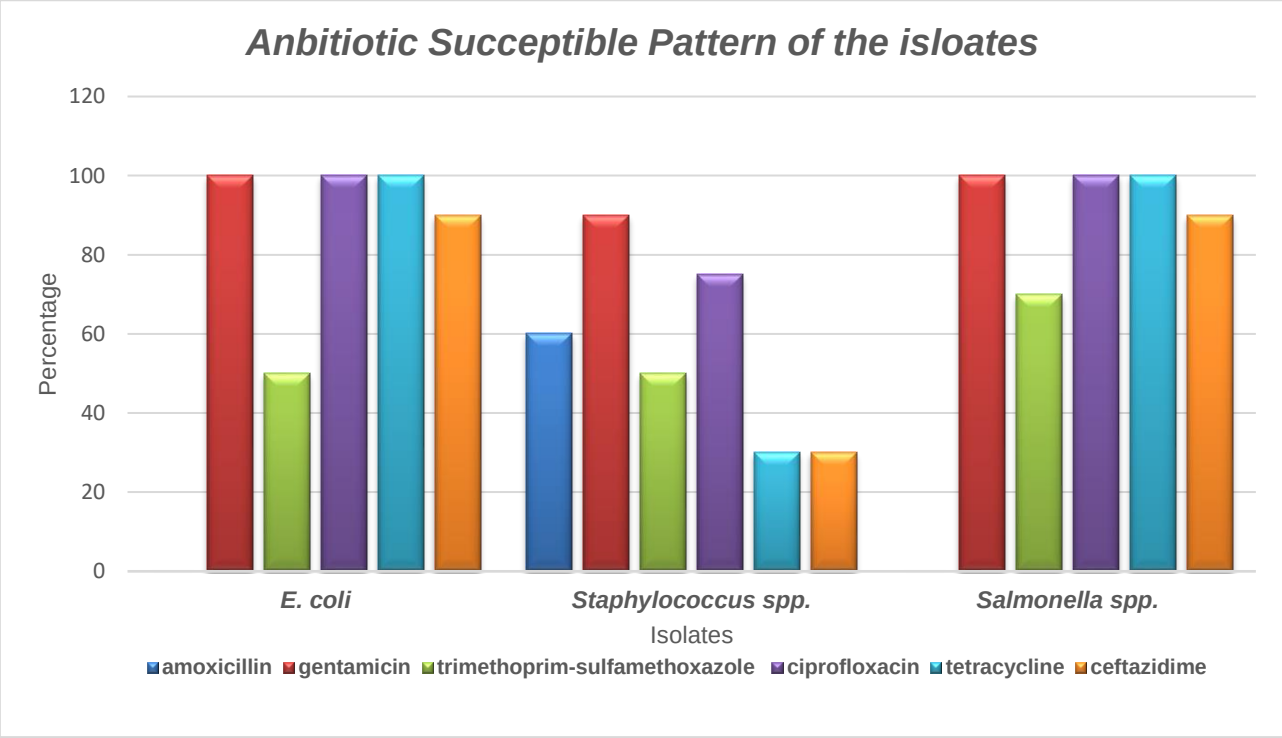

Fig. 2. Antibiotic Susceptibility pattern of the isolates

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. 1B). In this present study, all the salad samples were contaminated with the pathogens which are also observed to be present in 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 another study of Nepal (Poudel et al., 2020, Bezanson et al., 2008).

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 country, we need to focus on implementing hygiene measures, because frequent and overuse 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 countries 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. However, the major limitation of this study was the need for molecular characterization of the isolates.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/128240