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Isolation, identification and antibiogram of *Staphylococcus aureus* and *Escherichia coli* from salad vegetable

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ABSTRACT

Ready-to-eat foods, such as salad vegetables, often consist of raw ingredients from diverse sources and undergo extensive handling, making them vulnerable to microbial contamination from both human and environmental sources throughout production, harvesting, and handling before reaching the consumer, thereby increasing the risk of pathogenic microorganisms being present. This study assessed the microbial quality of restaurant salads in Sylhet and their role in spreading antibiotic-resistant bacteria. Samples from three categories of restaurants showed the presence of Staphylococcus aureus and *E. coli*. Initially, samples were cultured on nutrient agar, then on selective media. The appearance of yellow colonies and a green metallic sheen indicated the presence of Staphylococcus aureus and E. coli respectively. Antibiotic testing revealed that approximately 66.66% of Staphylococcus aureus isolates were highly resistant to Cefalexin but still sensitive to Gentamicin. All *E. coli* isolates exhibited strong resistance to both Cefalexin and Tetracycline, yet remained entirely sensitive to Gentamicin. Notably, no significant differences in antibiotic resistance patterns were observed among restaurants classified as low, medium, or high standard. The detection of multidrug-resistant bacteria is concerning, as it suggests that commonly used antibiotics may become less effective for treating infections caused by these organisms. This study emphasizes the critical importance of implementing strict hygiene protocol and comprehensive biosecurity practices at every stage- from farm to food distribution to reduce the risk of transmission to consumers.

1. Introduction

Salad vegetables are one kind of nutritious food that consist of a mixture of vegetables. A mixture of vegetable includes Green Chilies, Lettuce, Tomato, Carrots, Cabbage and Cucumber. These ingredients are packed with important nutrients, providing a low-calorie source of complex carbohydrate, vitamins, minerals, dietary fiber, antioxidants, and other beneficial plant compounds essential for good health (Rajvanshi, 2010). Eating vegetables regularly has been linked to a lower risk of heart disease, stroke, and some types of cancer (Pathak, 2022). The combined form of this food, called as one type of Ready-to-Eat foods, is minimally processed. But the use of untreated wastewater, irrigation water tainted by sewage, and inappropriate post-harvest handling can rise the possibility of contamination in salads. Besides, manure used for the improvement in crop production, can be a supplier of various pathogenic microbes (Simões et al., 2001; Beuchat, 2002).

Despite these health benrfits, foodborne illnesses connected to contaminated produce have increased over the past few decades. Eating raw or lightly cooked vegetables increases the risk of infection generally, are potential sources of pathogen transmission. Contamination with *E. coli* O157 has led to outbreaks of foodborne illness linked with the consumption of apple cider, lettuce, radishes, and salads made of a vegetable mixture. Fecal contamination of both home-cooked and commercially prepared foods is a major issue, often leading to outbreaks of stomach illnesses. In recent years, the rise and spread of antibiotic-resistant bacteria have become a global health concern (Harbarth et al., 2015). This problem is largely driven by the widespread and sometimes

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improper use of antibiotics in healthcare, farming, and food production. One major way antibiotic resistance develops and spreads is through eating foods that have been contaminated with antibiotics, which may further lead to food poisoning or allergies. During cultivation of crops, irrigated water and remained soil can be a source of micro-organism that resistance to antibiotics (Anokyewaa Appau & Ofori, 2024). In this study, the presence of bacteria and their potential susceptibility to antibiotics were investigated in the salads served in restaurants in the Sylhet Region of Bangladesh. Isolation of Staphylococcus aureus and *E. coli* was given particular attention as these bacteria are major public health concerns.

The objective of this study was to assess the antimicrobial pattern in vegetable salads served in restaurants located in Sylhet City, including the isolation and identification of resistant microorganisms. Along with salads, other accompaniments are served in restaurants, hotels, roadside eateries, and fast-food joints where the ingredients to make salads are locally available and demandable (Younus et al., 2019). Research has shown that E. coli can survive on minimally processed vegetables even in harsh environmental conditions (Kabir et al., 2014). The improper application of antibiotics, such as low and prolonged doses in livestock, makes sick animals on the farm increases the chances of spreading resistance throughout the food chain. This study focuses on characterizing the antibiotic resistance profile of Staphylococcus aureus and *E. coli* isolated from raw vegetable salads served in Sylhet city restaurants. We must persuade both the public and health professionals to recognize the serious dangers that arise from the misuse of antibiotics and lapses in food safety.

2. Materials and methods

2.1. Study area

The present experiment was carried out in the central laboratory of the faculty of Biotechnology and Genetic Engineering (FBGE), Sylhet Agricultural University, Sylhet, Bangladesh.

2.2. Sample collection and preparation

A Total of 24 samples were collected using well-labelled sterile polythene bags from 3 different categories (8 samples from each) restaurants/hotel (low, medium and high) of different location of Sylhet city. From each sample, 10 g was aseptically weighed and blended in a sterilized mortar and pestle under laminar air flow to prepare a stock solution. Seven sterile test tubes were taken, each containing 9 ml of sterile distilled water to carry out serial dilution. Every water sample was given a good shake before any dilution was made. The stock solutions were serially diluted up to 10-5 in accordance with the guidelines provided by the International Organization for Standardization (ISO, 1995). To isolate bacteria from salad vegetables, samples were incubated in nutrient media at 37°C for 24 hrs.

2.3. Isolation & identification of staphylococcus aureus and e. coli

Overnight culture of enriched media (turbidity of the media) was streaked onto Mannitol Salt Agar (MSA), Eosin Methylene Blue (EMB) Agar and MacConkey Agar, followed by 24hr incubation at 37°C. Positive isolates of *E. coli* grew with a distinctive metallic sheen color in EMB agar, and in MacConkey agar they gave bright pink colonies. Staphylococcus aureus was identified by observing yellow colonies on Mannitol Salt agar.
After incubation obtained, isolates were subjected to morphological & biochemical tests according to the method described by Microbiology Laboratory Manual (2020).

2.4. Morphological characterization and biochemical test of isolated colony

Microscopic analysis was done to characterize changes in size, shape, and morphology. The Gram stain is fundamental to the phenotypic characterization of bacteria.

The tests used for identifying bacteria species based on the differences in the biochemical activities of different bacteria are called as biochemical tests. Some types of biochemical tests performed in this experiment, according to the rules of the Microbiology Laboratory Manual (2020), were as follows: Catalase test (to accurately identify bacterial spores), Oxidase test (to get differences between different type of bacteria), Citrate test (to make alkaline medium which indicated by the change of color of the bromothymol blue indicator from green to blue) (MacWilliams, 2009), Indole test, Voges proskauer test & Methyl red test (to distinguish between several bacterial species, particularly within the Enterobacteriaceae family).

2.5. Antibiotics sensitivity test of isolated staphylococcus aureus and *E. coli*

In today's era, it has been a common occurrence to find bacteria resistant to a number of antimicrobial agents. Thus, these bacteria are converting into a more challenging form to combat them. In our experiment, bacterial isolates from salad samples were screened against three types of antibiotics - Tetracycline (30 µg), Gentamicin (10 µg) & Cefalexin (30 µg), to see whether these showed multidrug resistance properties or not. The disk diffusion method, also known as the Kirby-Bauer test, was followed for this step. According to Bauer et al. (1966), positive bacterial inoculum was spread on the plate that poured with Muller-Hinton agar medium. Then antibiotic discs were placed aseptically into the center of agar and incubated at 37°C within 15 minutes in an incubator for 16-18 hrs. After incubation, there were uniformly circular zones of inhibition on the surface. Zone diameters of each of the antibiotic discs were measured and the results were interpreted with the Clinical and Laboratory Standards Institute (CLSI) guidelines, 2023 (Aw, 1966; Rai et al., 2023). Standard reference strain for both types were used for comparison.

2.5. Data analysis

The collected data were analyzed using descriptive statistical methods, including calculation of prevalence and percentage resistance. Microsoft Excel was used to organize the data, calculate percentages, and generate tables and charts. The antibiotic susceptibility profiles were visualized through Excel-based bar and column charts, which clearly illustrate the distribution of resistant, intermediate, and susceptible isolates for Staphylococcus aureus and *E. coli*.

3. Results

Here a total of 24 samples were aseptically collected and prepared for testing Staphylococcus aureus and *E. coli*, and once an isolate was confirmed through morphological characteristics and biochemical identifications, it proceeded to antibiotic sensitivity testing.

3.1. Colony morphology and staining characteristics of isolates

Among them following incubation for 24 h, the large yellow or white colonies on nutrient rich agar and yellow colonies on mannitol salt agar confirmed the presence of Staphylococcus aureus. On nutrient agar, circular in shape, low convex elevation, smooth surface, grayish-white in color, and translucent structure was a primary hint for the presence of *E. coli*. The growth of smooth, green metallic sheen on EMB agar media and the development of pink colonies on MC Agar ensured *E. coli* positive cases. Following Gram staining, microscopic examination revealed the properties of *E. coli* (Gram-negative, pink, small rod, in chain) and S. aureus cells (Gram-positive, spherical and clusters resembling a bunch of grapes in appearance).

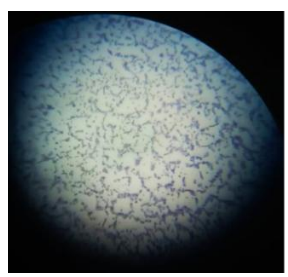
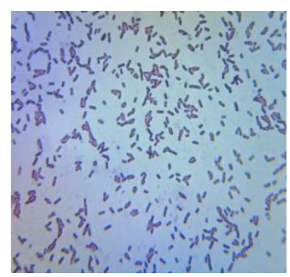

Figure 1: Gram staining result of *Staphylococcus aureus* (Left) and *E.coli* (Right)

S. aureus cells typically appear in clusters resembling a bunch of grapes and *E. coli* reveals pink, small rod, in chain under the light microscope following Gram staining (Figure 1).

3.2. Biochemical tests for the identification of *Staphylococcus aureus* and *E. coli*

Biochemical tests were done to confirm the isolates. Table 1 presents the isolates that were presumptively identified from the samples.

Table 1: Biochemical characterization of Staphylococcus aureus and *Escherichia coli* isolates

Presumptive organisms	Gram Staining	Biochemical tests					
		MR Test	VP Test	Oxidase Test	Catalase Test	Indole Test	Citrate Utilization Test
Staphylococcus aureus	+	+	−	−	+	−	+
Escherichia coli	−	+	−	−	+	+	−

+: Positive, −: Negative. MR: Methyl red, VP: Voges–Proskauer

3.3. Prevalence of staphylococcus aureus and e.coli isolates

In the prevalence study, out of total twenty four samples examined across three categories (eight samples per category), twenty four (100%) were found positive for Staphylococcus aureus. The presence of *E. coli* in salad samples was found 24 out of 24 samples (100%). Since each samples yielded one isolate of S. aureus and one isolate of *E. coli*, the total number of isolates obtained was 48. Both pathogens showed equal prevalence (100%), with no variation across categories, representing the highest possible detection range.

3.4. Antibiotic sensitivity

The antibiotic susceptibility profiles of Staphylococcus aureus and Escherichia coli isolates were determined using disk diffusion technique. Zones of inhibition were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Among twenty four isolates of S. aureus, twelve showed resistance against Tetracyclin (50%) and sixteen isolates showed resistance against Cefalexin (66.66%), while all remain susceptible to Gentamicin. In contrast, all isolates of *E. coli* showed resistance to both Tetracycline and Cefalexin but were fully susceptible to Gentamicin. This finding highlight that the isolation of multiple antibiotic resistant *S. aureus* and *E. coli* from salads poses a noteworthy threat to consumer health.

Figure 2: Antibiotic resistance profile of *Staphylococcus aureus* and *E. coli* isolates based on disk diffusion test.

Table 2: Percentage resistance of *Staphylococcus aureus* and *E. coli* isolates

Antibiotics Name	*Staphylococcus aureus* (n=24)			*Escherichia coli* (n=24)		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Tetracycline	(4) 16.66%	(8) 33.33%	(12) 50%	0%	0%	(24)100%
Cefalexin	0%	(8) 33.33%	(16) 66.66%	0%	0%	(24) 100%
Gentamicin	(16) 66.66%	(8) 33.33%	0%	(24) 100%	0%	0%

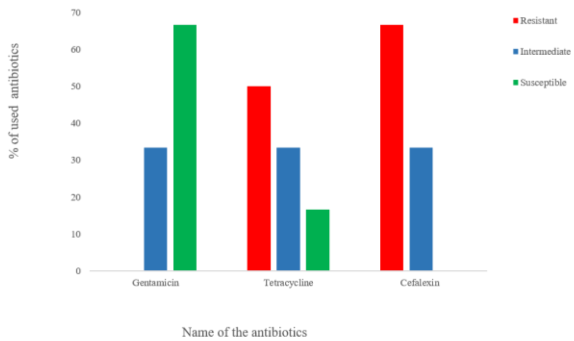

Figure 3: The figure showing the percentage resistance, intermediate response, and susceptibility of *S. aureus* isolates to Tetracycline, Cefalexin, and Gentamicin.

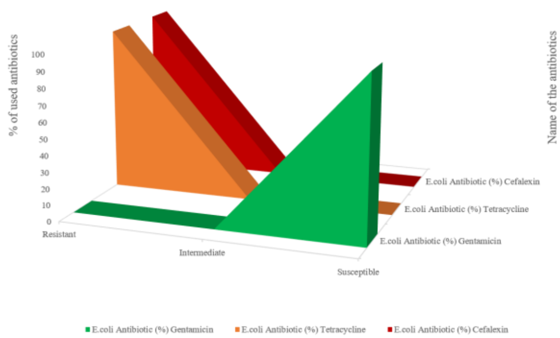

Figure 4: The percentage resistance, intermediate response, and susceptibility of *E. coli* isolates to Tetracycline, Cefalexin, and Gentamicin.

4. Discussion

Vegetables collected from direct field, especially those used in salad mixtures, have been intertwined in cases of food poisoning, posing significant health risks to consumers. This is primarily because most salad vegetables are consumed raw, without undergoing thermal processing or adequate washing (Castello et al., 2025). The high rate of bacterial contamination in salads we take as appetizer, observed in this study, can be attributed to several constituents, including the failure to adhere to basic sanitation protocols during the handling and processing of raw produce.

The detection of *S. aureus* contamination in salad samples is particularly serious concern that also discussed in (Sina et al., 2011). *S. aureus* is one of the prime causes of pyogenic infections and has been reported to exacerbate pre-existing superficial infections (Adegoke & Komolafe, 2009). A study by Jia et., al (Jia et al., 2024) also indicated a considerable contamination by S. aureus in each ingredient of salads. The study also highlighted the commonness of *E. coli* in salad samples, with *E. coli* being isolated from 100% of the samples analyzed and also discussed in (Rahman, 2015; Kabir et al.,2014).

Notable territorial variations in antibiotic resistance patterns were observed, as demonstrated in (Subhashini et al., 2014) , where *E. coli* isolates showed high resistance to Ampicillin, and Streptomycin, yet remained fully sensitive to Gentamicin. These regional differences emphasize the importance of location-specific data in understanding and managing antibiotic resistance. Integrating such data with genetic information is essential for effectively controlling outbreaks and optimizing antibiotic treatment strategies (Sarker et al., 2021). However, Gentamicin demonstrated the highest susceptibility against the isolates which supports the identification of another study conducted by Sultana, et al. in 2024 (Sultana et al., 2024).

The widespread development of antibiotic resistance, often linked to the overuse of antibiotics in livestock and their inclusion in animal feeds, causes a severe threat to both human and animal health (Kundu et al., 2021). In this study, Gentamicin resistance was found to be particularly high among the isolates, raising concerns about its uncontrolled use.

The investigation identified both gram-positive and gram-negative bacterial contaminants, including *S. aureus* and *E. coli*, in all salad samples from different graded restaurants. Contamination with *S. aureus* has been interconnected to the nasal carriage of the bacteria by food handlers or infected workers. Despite the resistance patterns observed, Gentamicin was identified as the most effective antibiotic against both *S. aureus* and *E. coli*. Antimicrobial resistance (AMR) is a complex issue impacting environmental, economic, biological, social, and developmental sectors, primarily driven by the inappropriate use of antimicrobial drugs. Resistance traits in pathogens can lead to ineffective treatment during foodborne disease outbreaks (Atia et al., 2022).

Identification of bacterial isolates in this study was based on colony morphology and biochemical tests, which limits accuracy of species determination and resistance claims. In addition, relatively small sample size reduces the generalizability of the findings. Reliance on phenotypic traits alone cannot confirm resistance mechanisms, underscoring the need for molecular approaches such as 16S rRNA sequencing and PCR assays in future work.

5. Conclusion

This study highlights concerning levels of contamination with Staphylococcus aureus and Escherichia coli in salads served at local fast-food outlets and restaurants in Sylhet, raising significant public health concerns. It also emphasizes the presence of antibiotic-resistant bacterial strains in salad vegetables sold in these establishments. Given that salads are typically served as appetizers without further cooking, it is crucial to thoroughly wash raw vegetables and soak them in food-grade antibacterial solutions for an appropriate duration to reduce microbial contamination and eliminate foodborne pathogens. The findings demonstrate that raw vegetable salads can harbor pathogenic bacteria capable of surviving in their uncooked state, posing a considerable risk of foodborne illness to consumers. To mitigate these risks, all restaurants should implement enhanced hygiene practices during food preparation, handling, and service. Strengthening food safety protocols and providing education to food handlers on proper hygiene and sanitation are essential measures to protect public health and reduce contamination in salads served by restaurants.

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References

Adegoke, A. A., & Komolafe, A. O. (2009). Multi-drug resistant Staphylococcus aureus in clinical cases in Ile-Ife, Southwest Nigeria. International Journal of Medicine and Medical Sciences, 1(3), 068-072.

Anokyewaa Appau, A. A., & Ofori, L. A. (2024). Antibiotic Resistance Profile of E. coli isolates from lettuce, poultry manure, irrigation water, and soil in Kumasi, Ghana. International Journal of Microbiology, 2024(1), 6681311.

Atia, A., Hosien, B., & Belhaj, H. (2022). Antimicrobial resistance in Libya: A systematic literature review of two decades. Biomedical and Biotechnology Research Journal (BBRJ), 6(4), 473-482.

Castello, A., Massaro, C., Seghers, E., Ferraro, C., Costa, A., Alduina, R., & Cardamone, C. (2025). Isolation and Molecular Characterization of Antimicrobial-Resistant Bacteria from Vegetable Foods. Pathogens, 14(7), 682.

Beuchat, L. R. (2002). Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Microbes and infection, 4(4), 413-423.

Harbarth, S., Balkhy, H. H., Goossens, H., Jarlier, V., Kluytmans, J., Laxminarayan, R., Saam, M., Van Belkum, A., Pittet, D., & participants, W. H.-A. I. R. F. (2015). Antimicrobial resistance: one world, one fight! Antimicrobial Resistance and Infection Control, 4(1), 49.

Jia, K., Qin, X., Bu, X., Zhu, H., Liu, Y., Wang, X., Li, Z., & Dong, Q. (2024). Prevalence, antibiotic resistance and molecular characterization of Staphylococcus aureus in ready-to-eat fruits and vegetables in Shanghai, China. Current research in food science, 8, 100669.

Kabir, A., Das, A. K., & Kabir, M. S. (2014). Incidence of antibiotic resistant pathogenic bacteria in vegetable items sold by local and super shops in Dhaka city. Stamford Journal of Microbiology, 4(1), 13-18.

Kundu, T., Rumi, N. A., Hossain, M. K., Rahman, M. S., Hossain, M. M. K., & Halder, J. (2021). Isolation of multidrug-resistant Escherichia coli from turkeys in Dinajpur, Bangladesh, and their antibiogram profile. Journal of advanced veterinary and animal research, 8(1), 64.

MacWilliams, M. (2009). Citrate test protocol. American Society for Microbiology Laboratory Protocols. In.

Pathak, N. (2022). Role of vegetables in human nutrition and disease prevention. The Pharma Innovation Journal, 11(5), 1745-1748.

Rahman, M. M. (2015). Effectiveness of different sanitizers in inactivating E. coli O157: H7 in Tomato and Cucumber. Journal of Food and Nutrition Sciences.

Rai, S., Dash, D., & Agarwal, N. (2023). Introducing the new face of CLSI M100 in 2023: An explanatory review. Indian Journal of Medical Microbiology, 46, 100432.

Rajvanshi, A. (2010). Bacterial load on street vended salads in Jaipur City, India. Internet Journal of Food Safety, 12(1), 136-139.

Sarker, M. S. A., Shahid, M. A. H., Hoque, M. N., Sarker, M. A., Rahman, M. B., & Islam, S. S. (2021). The rich mapping: Be a supplementary approach for anthrax control at community level. Journal of Advanced Veterinary Research, 11(1), 41-46.

Simões, M., Pisani, B., Marques, E. G. L., Prandi, M. A. G., Martini, M. H., Chiarini, P. F. T., Antunes, J. L. F., & Nogueira, A. P. (2001). Hygienic-sanitary conditions of vegetables and irrigation water from kitchen gardens in the municipality of Campinas, SP. Brazilian Journal of Microbiology, 32, 331-333.

Sina, H., Baba-Moussa, F., Kayodé, A., Noumavo, P., Sezan, A., Hounhouigan, J., Kotchoni, S., Prévost, G., & Baba-Moussa, L. (2011). Characterization of Staphylococcus aureus isolated from street foods: Toxin profile and prevalence of antibiotic resistance. Journal of Applied Biosciences, 46, 3133-3143.

Subhashini, R., Suganthi, R., Krithika, A., Poorani, S., Gopakumar, C., & Alliddawi, I. M. (2014). Screening and sequencing of antibiotic resistant microorganisms from street food. Int. J. Pharm. Sci. Rev. Res, 24(1), 224-229.

Sultana, R., Alam, S. T., Akter, S., Mia, P., Nabila, S. S., Tasnim, S., Ferdous, M., Islam, M. R., Khan, M. S., & Akash, A. A. F. (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), 92-99.

Younus, M. I., Sabuj, A. A. M., Haque, Z. F., Sayem, S. M., Majumder, S., Parvin, M. S., Islam, M. A., & Saha, S. (2019). Microbial risk assessment of ready-to-eat mixed vegetable salads from different restaurants of Bangladesh Agricultural University campus. Journal of advanced veterinary and animal research, 7(1), 34.