Available online at www.most.gov.bd
Volume 06, Issue 01, Pages: 45–51, 2024
DOI: https://doi.org/10.3329/jscitr.v6i1.77374

Journal of Science and Technology Research
Ministry of Science and Technology
Government of the People's Republic of Bangladesh
Bangladesh Secretariat, Dhaka

Antimicrobial Pattern of Foodborne Bacterial Hazards Isolated from Vegetables in Dhaka City

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Abstract

The prevalence of undesirable microbial contaminants in fresh vegetables, often consumed raw as salad vegetables, is a concern for the global public health community. In this study, samples from different local markets in Dhaka were collected to evaluate the microbiological quality and safety of four raw salad vegetables, namely, lettuce, tomato, cucumber, and coriander leaves. Microbiological analyses revealed a higher total aerobic bacterial count (3.10 to 8.71 log CFU/g) and coliform count (non-detectable to 7.61 log CFU/g) regardless of RSV type. In all of the examined shop types, foodborne pathogens like *Escherichia coli* and *Salmonella* spp. were found in more than 60% of the analyzed vegetable samples, and the isolated *E. coli* and *Salmonella* spp. were found to be completely resistant to 3 different antibiotics (Amoxicillin, Erythromycin, and Rifampicin) and intermediately resistant to 2 other antibiotics (Bacitracin and Novobiocin). Therefore, the study findings revealed that the presence of multidrug-resistant *E. coli* and *Salmonella* spp. in fresh vegetable samples is a cause for worry when consumed raw. Controlled environment production approaches, such as indoor farming of vegetables and washing with plenty of fresh water before consumption, can help to reduce pathogen and antimicrobial resistance bacteria.

Received: 01.02.2024
Revised: 08.06.2024
Accepted: 22.06.2024

Keywords: Antimicrobial Resistance, Foodborne Pathogen, Raw Salad Vegetables, Microbiological Quality, Multidrug-resistant Bacteria.

Introduction

Foodborne illnesses associated with fresh vegetables pose significant challenges to public health and economic stability (Callejón *et al.*, 2015; CSPI, 2014). Many nations, including Bangladesh, are experiencing an increase in food-borne illnesses associated with vegetables (Noor and Feroz, 2016). Contamination can occur in all vegetables sold in public markets, supermarkets, and even on roadside stalls, posing serious health risks to customers (Lagerkvist *et al.*, 2013). Poverty and a lack of hygiene and sanitation awareness contribute considerably to the burden in developing nations such as Bangladesh (Chowdhury *et al.*, 2013). The risk of microbial contamination is very high when vegetables are cultivated outdoors, so hygiene precautions are critical throughout the entire production and unsanitary supply chain. Microorganisms can contaminate fruits and vegetables at any point in the production process, posing a substantial risk to consumer health (Balali *et al.*, 2020). The risk is compounded when product is consumed without sufficient washing, as well as a lack of knowledge among farmers and customers about the importance of exercising comprehensive cleanliness. In Bangladesh, the horticultural sector suffers major food safety difficulties due to post-harvest handling, poor processing practices, no use

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of sanitizer, inadequate packaging, transportation challenges, and inadequate personal hygiene practices among populations (Ahmed *et al.*, 2017; Leone *et al.*, 2022).

Food-borne infections are the major public health concern worldwide, accounting for an estimated 1.9 million deaths each year (Jahan, 2013). About one-third of the world's population, including those in industrialized countries, suffers a foodborne illness every year (Akhtar *et al.*, 2014). This problem is especially acute in resource-constrained countries due to inadequate food handling hygiene standards. Parasites, viruses, and fungus can all cause vegetable contamination and disease, but bacteria are responsible for the majority of foodborne outbreaks (Founou *et al.*, 2021). Post-harvest handling, processing areas, and consumer activity are all responsible for the risk of bacterial contamination (Ahmed *et al.*, 2014). Nowadays, it is commonly accepted that consuming raw vegetables increases the chance of developing bowel infections. On the other hand, because of the emergence and rapid spread of antibiotic resistance in humans, animals, and the environment, pathogenic bacteria that frequently infect vegetables are recognized as a global health concern (Hölzel *et al.*, 2018). There are concerns about the spread of antibiotic-resistant bacteria from fresh vegetables into the general population (Rupa *et al.*, 2012). Nonetheless, there has been limited study in low-income countries on foodborne pathogens and their antibiotic resistance patterns in humans, animals, food, and the environment. To the best of our knowledge, there is no previous research on methicillin-resistant staphylococci, beta-lactamase-producing Enterobacteriaceae, or multidrug-resistant bacteria in commonly consumed vegetables. Furthermore, there are few market-level microbiological quality studies for vegetables widely consumed in Bangladesh. As a result, the purpose of this study was to evaluate bacterial contamination levels and antibiotic resistance patterns in regularly consumed market vegetables in Dhaka city.

Materials and Methods

Selection of Markets for Sample Collection

The sampling points targeted the marketplaces featured chain stores, wholesale stores, and retail stores that made sure salad vegetables were bought by families with high, medium, and low incomes. A total of twelve marketplaces were chosen based on their level of popularity within the Dhaka metropolitan region. Three markets, namely Agora (Gulshan-2), Swapno (Banani), and Minabazar (Dhanmondi), were selected as the most-liked chain stores out of them. Six marketplaces, namely, New Market, Mohammadpur Krishi Market, Mirpur-1, and Santinagar bazaar, Mohakhali, and Khilkhet and, were selected as prominent retail markets, while three, namely Kawran bazar, Shyambazar, and Jatrabari, were designated as popular wholesale markets.

Inclusion and Exclusion Criteria of Vendors

The vendor inclusion criterion was based on age and length of operation. Vendors with more than five years of experience and ages ranging from 25 to 45 were included. When multiple vendors were selling the same vegetable, only those who matched the inclusion criteria and agreed to participate in the study were included. When multiple vendors from the same market agreed to participate, independent vegetable samples were obtained for the study. To accommodate as many vendors as feasible, our poll only included one vegetable kind from a single supplier.

Sample Collection

Samples were collected by skilled and experienced MS students from the Food, Nutrition and Agriculture Research Laboratory (FNARL), Center for Advanced Research in Sciences, University of Dhaka. Samples were collected from February to April 2023. A total of 120 fresh vegetables samples were collected, as they are typically purchased by people. Samples of lettuce (n=30), tomato (n=30), cucumber (n=30), and coriander leaf (n=30) were obtained from the marketplace vendors of Dhaka city. All the samples were collected in sterile sample collection bags and kept in a cold box and transported to the laboratory within 1 to 2 hours of collection by maintaining temperature. The samples were given laboratory code marks, and then separated into two equal halves (one for microbiological analysis and one for storage). Finally, the samples were refrigerated (2 to 8 ^{o}C) until the analyses was completed.

Quality Compliance Laboratories (QCL)

This experiment was carried out in collaboration with WAFFEN Research Laboratory, an accredited laboratory located in Dhaka. A systematic random sampling approach was utilized, and one-third of the total samples were cross-checked in WAFFEN Research Laboratory to determine the quality of analyses and variances.

Microbiological Analysis

The samples were weighed (25 g) and placed into sterile stomacher bags (Japan). After adding the 90 mL of sterile normal saline, the stomacher bags were stomached for 90 seconds at 230 rpm. On both selective and non-selective agar plates, the processed samples were surface plated. Decimal serial dilution were done where necessary and 100 µL of stomacher-treated samples were surface plated onto Tryptic Soya Agar (Oxoid, England) for the determination of total aerobic bacterial count (APC); Sorbitol MacConkey Agar (Oxoid, England) for total coliform count (TCC); EC agar (Nissui, Japan) for *E. coli* count; and Bismuth Sulfite Agar (Oxoid, England) for *Salmonella* spp. count. Moreover, the samples were enriched by inoculation of the samples into tryptic soy broth (37 ºC for 24 to 48 hours) followed by streaking on selective media to confirm the presence or absence of *E. coli* and *Salmonella* spp. The biochemical test API 20E was used to confirm the presumed bacteria cultivated in the selectiveand non-selective medium. Log CFU/g was used to record all of the plate counts data.

Antimicrobial Susceptibility Testing

The antibiogram profile of randomly selected isolates was performed against 18 different antibiotics using disc diffusion technique (Bauer *et al.*, 1966). The following antibiotic discs from Oxoid (Basingstoke, England) was used: Kanamycin (30 µg), Bacitracin (10 µg), Novobiocin (30 µg), Polymyxin B (300 µg), Erythromycin (15 µg), Rifampicin (5 µg), Ciprofloxacin (5 µg), Azithromycin (15 µg), Streptomycin (10 µg), Tetracycline (30 µg), Nalidixic acid (30 µg), Aztreonam (30 µg), Nitrofurantoin (300 µg), Ampicillin (10 µg), Chloramphenicol (30 µg), Piperacillin (110 µg), Ceftazidime (30 µg), Amoxicillin (10 µg). The diameters of inhibition zones were measured against the Clinical Laboratory Standards Institute (CLSI) standard (Bruna *et al.*, 2022).

Statistical Analyses

Following collection and analyses, reported plate count data represented the mean values obtained from three individual trials, each of which was derived from duplicated samples. The data were subjected to variance analysis using the Microsoft Excel program (Redmond, Washington, DC, USA). Significant changes in all selective and non-selective plate count data were determined by the least significant difference at the 5% level of significance.

Results and Discussion

The microbiological analysis was done according to the ISO standard methods, and the average microbiological counts were presented in Table 1 with statistical analyses of 5% level of significance. Microbiological analysis showed that regardless of market location, a larger number of APC was found, ranging from 4.07 to 7.03 log CFU/g in lettuce samples, 3.10 to 6.90 log CFU/g in tomato samples, 3.85 to 8.71 log CFU/g in cucumber samples, and 3.98 to 8.45 log CFU/g in coriander leaf samples. Total coliform count ranging from non-detectable to 6.87 CFU/g in lettuce; 2.20 to 5.00 log CFU/g in tomato; non-detectable to 7.61 log CFU/g in cucumber; and 3.02 to 7.32 log CFU/g in coriander leaf. The yeast and mold count ranged from 1.07 to 4.10 log CFU/g in lettuce; 1.80 to 4.30 log CFU/g in tomato; 1.20 to 4.10 log CFU/g in cucumber; and 2.90 to 6.70 log CFU/g in coriander leaf (Table 1). *E. coli* was identified in 30% of the lettuce samples, while *Salmonella* spp. was detected in 10% of the lettuce samples. On the other hand, 36.7% of the cucumber samples were found to be contaminated with *E. coli*, but no *Salmonella* spp. contamination was evident. The coriander leaf was found to be significantly polluted (86.7%) with *E. coli*, whereas 6.7% of the coriander samples were found contaminated with *Salmonella* spp. Approximately 76.7% of tomato samples were contaminated with *E. coli*, and 20% of tomato samples were found polluted with *Salmonella* spp. Regardless of the vegetables studied, 57.5% of the total samples were polluted with *E. coli* and 9.2% with *Salmonella* spp.

Table 1. Average (Mean±SD) microbial load and presence of safety indicator bacteria in lettuce, tomato, cucumber, and coriander leaf

	Average microbial population (log CFU/g)				
Types of vegetables (n)	**TABC**	**TCC**	**Y&M**	***Presence of E. coli***	***Presence of Salmonella* spp.**
Lettuce (30)	5.72±0.29	4.15±0.21	2.57±0.13	9 (30)	3 (30)
Tomato (30)	5.36±0.27	3.81±0.19	3.40±0.17	23 (30)	6 (30)
Cucumber (30)	5.86±0.29	3.68±0.18	2.92±0.15	11(30)	0 (30)
Coriander leaf (30)	6.39±0.32	5.09±0.25	4.56±0.23	26 (30)	2 (30)

Detection limit: 1.0 log CFU/g; TABC = total aerobic bacterial count; TCC = total coliform count; Y&M = yeast and mold count. Parenthesis indicates the number of samples analyzed. *E. coli* and *Salmonella* spp. are considered as safety indicator bacteria.

Among the 69 *E. coli* isolates, randomly selected 19 *E. coli* isolates and all *Salmonella* spp. (11 isolates) recovered from lettuce, tomato, cucumber, and coriander leaves were subjected to an antibiogram investigation against 18 regularly used antibiotics. Figure 1 shows the percentage of total resistant, intermediately resistant, and sensitive patterns of isolated *E. coli* (A) and *Salmonella* spp. (B) to 18 commonly used antibiotics and the result adopted from the inhibition zone diameter of disc diffusion technique. Moreover, Figure 2 revealed that all *E. coli* (n=19) and *Salmonella* spp. (n=11) isolates were completely resistant to amoxicillin, erythromycin, and rifampicin. On the other hand, 14 of 19 *E. coli* isolates and 9 of 11 *Salmonella* isolates were found to be intermediately resistant to novobiocin and bacitracin antibiotics. 45.5% of *Salmonella* isolates were intermediately resistant to streptomycin and azithromycin, whereas 42.1% of *E. coli* isolates were resistant to ampicillin and 36.8% were intermediately resistant to nitrofurantoin.

In Bangladesh, vegetables are consumed in larger quantities than grains and their byproducts (Timsina *et al.*, 2018). Additionally, the components of vegetables contribute to health nutrition when consumed (Liu, 2003). The microbial quality and safety of the fresh vegetables including lettuce, tomato, cucumber and coriander leaves were evaluated in this study. The microbiological data revealed that 70% of the vegetables tested exceeded the permitted level set by the Bangladesh Food Safety Authority (BFSA) for fresh fruits and vegetables (BFSA, 2022). The presence of *E.coli* and *Salmonella* spp., in any food suggests fecal contamination and is not permitted for human consumption; however, in this study, regardless of the vegetables studied, 57.5% of the total samples were found contaminated with *E. coli* and 9.2% with *Salmonella* spp., implying that these vegetables can cause diarrhea in humans, if consumed raw. Furthermore, *E. coli* and *Salmonella* spp. isolated from these vegetables were found completely resistant to 3 antibiotics and intermediately resistant to 2 additional antibiotics exacerbated the situation. Although cleaning and sanitation is standard postharvest handling practices to ensure the quality and safety of vegetables, sanitizing fruits and vegetables is not widespread in Bangladesh, thus introducing unsafe and poor-quality vegetables in the marketplaces (Castro-Ibáñez *et al.*, 2017). Hence, pathogenic microorganisms must be removed from the surfaces of the vegetables before they are packed and sold in the market.

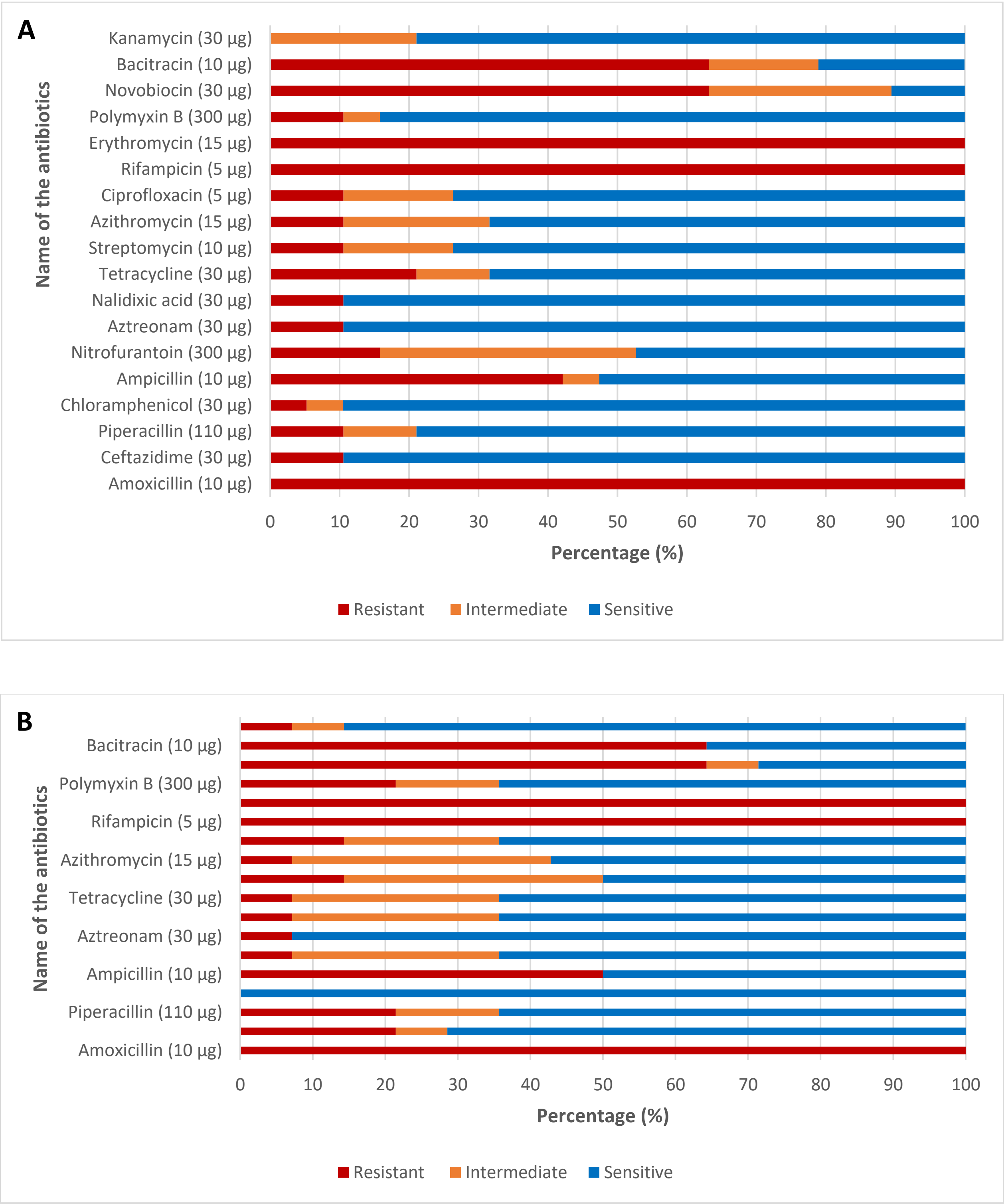

Figure 1. The percentage of total resistant, intermediately resistant, and sensitive patterns of isolated *Escherichia coli* (A) (n = 19) and *Salmonella* spp. (B) (n = 11) to 18 commonly used antibiotics.

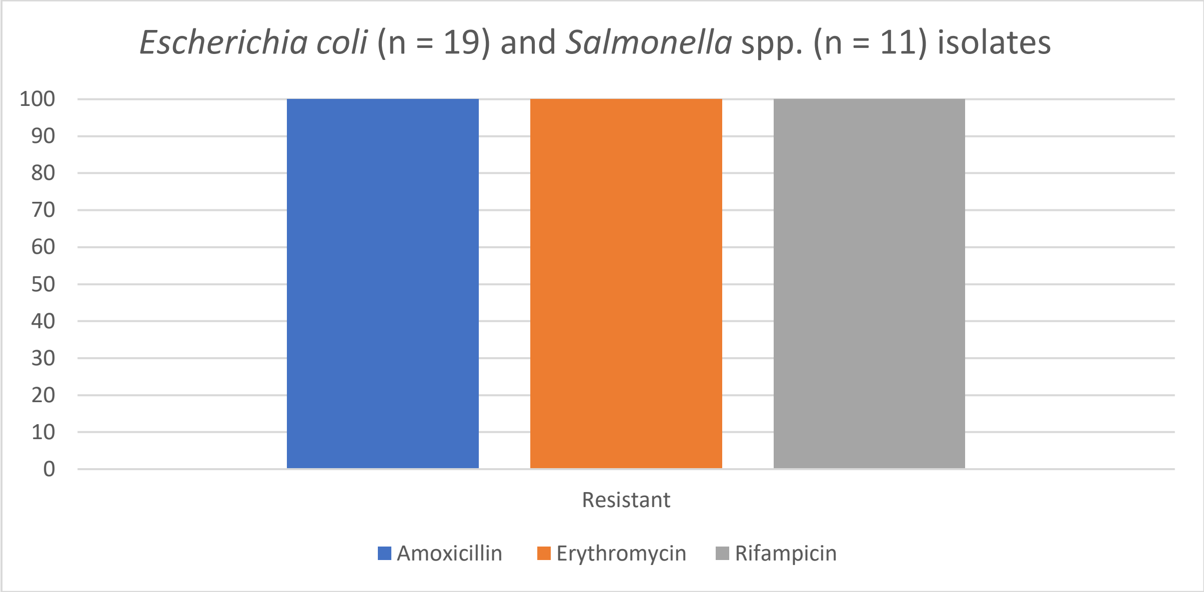

Figure 2. Completely resistant profiles of *Escherichia coli* and *Salmonella* spp. to antibiotics

Conclusions

The study's findings revealed that fresh vegetables sold in marketplaces are of poor quality, with the prevalence of multidrug-resistant pathogenic bacteria such as *E. coli* and *Salmonella* spp. As a result, good agricultural practices (GAP), as well as the use of sanitizers, post-harvest processing facilities, and the introduction of transport utensils, storage boxes, and exhibiting baskets, could be able to provide safe fresh vegetables in the marketplaces.

Acknowledgements

The authors would like to thank Ministry of Science and Technology, Government of the People's Republic of Bangladesh for financial assistance (SRG-221211, 2022–2023).

Declaration

The authors declare that there is no conflict of interest in the content of this manuscript and all the authors agreed for its submission in this Journal.

Authors' Contributions

SZ was in charge of sample collection, methodology, data analyses, and the initial draft, while MLB resourced, developed, and supervised the project, performed data analysis, wrote, reviewed, and edited the manuscript, and was in charge of fund aquisition.

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