International Conference on Sustainable Biotechnology (ICoSBi)
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Identification of Antibiotic-Resistant Bacteria in Ready-to-Eat Salads Available in Supermarkets in Thalawathugoda Area, Sri Lanka

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Abstract

The increasing demand for ready-to-eat (RTE) salads has raised concerns about food safety due to the potential for contamination by antibiotic-resistant bacteria. This study focused on the identification of antibiotic-resistant bacteria and their resistance profiles to discover resistant bacterial strains prevalent in RTE salads sold in supermarkets in Thalawathugoda area, Sri Lanka. To confirm the presence of pathogens, five different types of RTE salads were sampled and put through molecular analysis. These included Antibiotic Sensitivity Testing (ABST), DNA extraction, Polymerase Chain Reaction (PCR), Agarose gel electrophoresis, and Sanger sequencing. Antibiotic-resistant bacteria were found in S1 – Greek salad (GS), S3 – Potato salad (PS), S4 – Fresh Mix salad (FS), and S5 – Chicken potatoes and pineapple salad (CS), out of the five samples, according to the results. Amoxicillin displayed the strongest resistance in several samples, while ciprofloxacin was the most effective antibiotic according to ABST sensitivity patterns. Bacteria isolated from S2 - Apple salad (AS) showed sensitivity to each of the three tested antibiotics, suggesting that it is a high-quality sample with little chance of contamination by antibiotic resistant pathogenic bacteria. *Staphylococcus* spp. and *Escherichia coli* were detected in tested samples using PCR and agarose gel electrophoresis. *Klebsiella quasipneumoniae* was identified in S4 through Sanger sequencing, indicating the need for careful handling. The research highlights the significance of maintaining proper hygiene and implementing monitoring practices across the entire food supply chain, from production to retail, to minimize the risk of contamination in RTE salads. Further research and regular microbiological testing are recommended to ensure food safety and protect the health of customers.

Keywords: *Antibiotic Sensitivity Testing (ABST), Escherichia coli., Ready-to-eat (RTE) salads, Klebsiella quasipneumonia, Staphylococcus* spp.

https://doi.org/10.54389/YHRS4929

Introduction

The increasing preference for ready-to-eat (RTE) salads among customers has raised concerns about food safety, especially the possibility of antibiotic-resistant bacteria contaminating food. These salads, which are primarily composed of uncooked vegetables and other ingredients, have become more popular because of their simple preparation and beneficial health qualities. In the past decade, the requirement for ready-to-eat (RTE) salads and leafy green vegetables has grown as more consumers embrace healthier lifestyle habits (Taban, et al., 2011). The RTE salads are susceptible to microbial contamination from a variety of sources, such as soil, water, human interaction, and cross-infection during manufacture, because they are consumed raw without additional processing or heating (Łepecka, et al., 2022). This has raised concerns, especially concerning the potential presence of antibiotic-resistant microbes in these foods. Antibiotic resistance is a critical global health challenge since the emergence of resistant bacterial strains makes treating bacterial illnesses more difficult. The extensive use of antibiotics in agriculture and food production, especially in livestock, has accelerated the development of resistant microorganisms (Okaiyeto, et al., 2024). When found in food products like RTE salads, bacteria like *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*), which frequently develop resistance to commonly used antibiotics, can pose a considerable risk to human health (Söderqvist, et al., 2016). Given the continued growth of antibiotic resistance, the presence of these microbes in food products must be closely checked. This study is especially relevant in Sri Lanka, where only a limited number of previous studies focused on microbial contamination and antibiotic resistance in RTE salads. Research and public knowledge of the microbiological safety of these convenience foods are noticeably lacking, despite their increasing usage. A thorough and reliable system for monitoring food safety is also lacking in Sri Lanka, particularly for fresh produce that is sold in supermarkets. Recent occurrences of food poisoning connected to contaminated food items highlight the critical need for research and local data. In Sri Lanka, bacterial diarrhea and hepatitis A, *Salmonella* infections, contamination by *Listeria monocytogenes*, and *Vibrio cholerea* are significant causes of food-borne illness (Munasinghe, et al., 2015).

This study aimed to determine the presence of antibiotic-resistant bacteria in ready-to-eat (RTE) salads sold in supermarkets of Thalawathugoda area in Sri Lanka using methods like PCR, Sanger sequencing, and antibiotic sensitivity testing. The findings will offer insights into bacterial contamination and resistance patterns, emphasizing the need for improved food safety regulations to prevent the spread of antibiotic-resistant bacteria through the food chain.

Materials and Methods

This study examined five different varieties of RTE salads (S1, S2, S3, S4, and S5) that were purchased from two prominent Sri Lankan supermarket chains to assess the degree of antibiotic-resistant bacterial contamination. Salads were homogenized and processed aseptically to prevent cross-contamination and inoculated in sterile Luria Bertani (LB) agar (Nipa, et al., 2011) using the spread plate method. After 24 hours of incubation at 37 °C, colonies were observed. Sub-culturing was performed using the streak plate method to isolate morphologically different colonies. Sub-culturing was not done for S1 and S5 due to the lack of morphological variation. Antibiotic Sensitivity Testing (ABST) was used to evaluate resistance to Amoxicillin (10 μg/mL), Ciprofloxacin (5 μg/mL), and Gentamicin (10 μg/mL). Zones of inhibition were measured following CLSI guidelines (Bauer, n.d.). A sample is categorized as resistant, intermediate, or sensitive using this qualitative method (Galhano, et al., 2021). DNA was extracted using the boiling method (Dashti, et al., 2009). The primers used for PCR amplification targeted the 16S rRNA gene for both *Staphylococcus* spp. and *E. coli*. Species-specific primers for *Staphylococcus* (Stap) were used for PCR detection. The expected band size was 367bp (Martineau, et al., 2001). PCR was performed on extracted DNA samples of S1, S3-B and S5. Each reaction mixture (25 μL) contained 5 μL of the DNA template, 0.5

μL of Taq DNA polymerase, 2.5 μL of 10× PCR buffer, 0.5 μL of 10mM dNTPs, 0.5 μL of 10 μM Stap forward primer, 0.5 μL of 10 μM Stap reverse primer, and 15.5 μL of distilled water. The thermal cycle included an initial denaturation at 94 °C for 5 minutes, followed by 40 cycles of denaturation (94 °C, 30 s), annealing (55 °C, 30 s), and extension (72 °C, 30 s), with a final extension at 72 °C for 5 minutes. Species specific primers for the *E. coli* were used for PCR detection. The expected band size was 101 bp (Spano, et al., 2005). PCR was performed on extracted DNA samples of S1, S3-A, S3-B, S3-C, S5. Each reaction mixture (25 μL) contained 5 μL of the DNA template, 0.5 μL of Taq DNA polymerase, 2.5 μL of 10 x PCR buffer, 0.5 μL of 10mM dNTPs, 1 μL of 10 μM *E. coli* forward primer, 1 μL of 10 μM *E. coli* reverse primer, and 14.5 μL of distilled water. The thermal cycle included an initial denaturation at 95 °C for 5 minutes, followed by 30 cycles of denaturation (94 °C, 30 s), annealing (55 °C, 30 s), and extension (72 °C, 40 s) with a final extension, at 72 °C for 10 minutes. The PCR reaction for both sets of primers was performed in a programmable PCR Thermal Cycler. 2% agarose gel electrophoresis confirmed the presence of target bacterial genes. Sanger sequencing was performed by Macrogen, South Korea, to identify the species that could not be identified by the above molecular analysis.

Results

The overall results of ABST showed that ciprofloxacin was efficient against bacterial isolates from all salad samples, but amoxicillin and gentamicin had different levels of efficacy. PCR was used to identify *E. coli* and *Staphylococcus* spp. in samples S1, S3, and S5. Notably, Sanger sequencing revealed that sample S4 contained *Klebsiella quasipneumoniae*. These results show that different varieties of ready-to-eat salads have a variety of microorganisms, including coliform and antibiotic-resistant bacteria.

Table 1: *Summary of overall results of the study*

Sample name		**S1 (GS)**	**S2 (AS)**		**S3 (PS)**			**S4 (FS)**		**S5 (CS)**
Sub culturing		-	S2-A	S2-B	S3-A	S3-B	S3-C	S4-A	S4-B	-
ABST results (Effective antibiotics)	Ciprofloxacin	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Gentamicin	-	✓	✓	-	-	✓	✓	✓	✓
	Amoxicillin	-	✓	✓	-	✓	-	-	✓	-
Species identified through PCR	*Staphylococcus*	✓	-	-	-	✓	-	-	-	✓
	Escherichia coli	✓	-	-	✓	✓	✓	-	-	✓
Species identified through Sequencing	*K. quasipneumoniae* SUB15338200 4A PV668983	-	-	-	-	-	-	✓	-	-

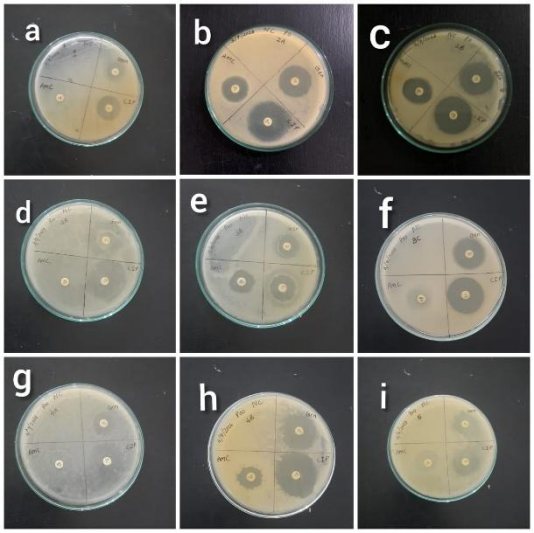

Figure 1: *ABST Results (a) S1 sample (b) S2 -A Subculture (c) S2 – B Subculture (d) S3 -A Subculture (e) S3 -B Subculture (f) S3 -C Subculture (g) S4 -A Subculture (h) S4 -B Subculture (i) S5 Sample*

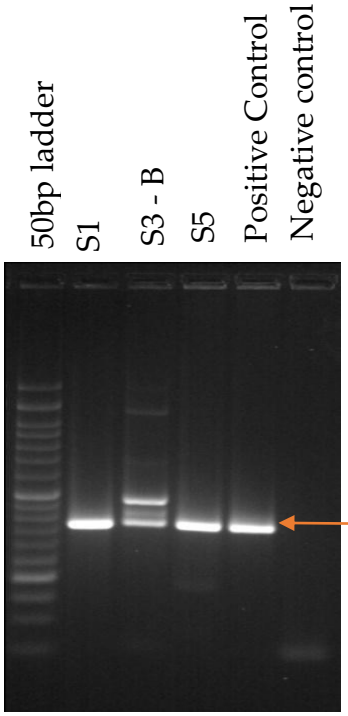

Figure 2: *PCR amplification of the target gene specific for Staphylococcus* spp. *Bands at 367 bp confirm the presence of Staphylococcus spp. in the respective samples.*

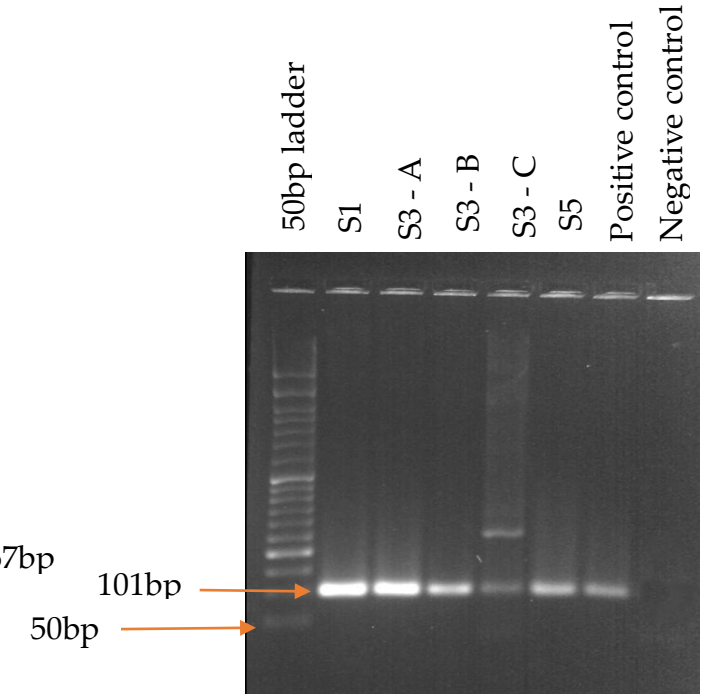

Figure 3: *PCR amplification of the target gene specific for E. coli Bands at 101 bp confirm the presence of E. coli in the respective samples.*

Discussion

Ready-to-eat (RTE) salads have become increasingly popular in Sri Lanka due to their convenience and perceived health benefits. However, they may harbor harmful, antibiotic-resistant bacteria due to their inadequate preparation and incomplete cooking. The danger of foodborne infections continues to rise in the absence of thorough local studies and appropriate food safety monitoring systems.

The outcomes of this study give important insights into the variety and prevalence of bacteria resistant to antibiotics in ready-to-eat (RTE) salads that are often sold in Sri Lankan supermarket chains. Such findings are consistent with previous studies showing that minimally processed foods, such as salads, can act as carriers of bacteria resistant to antibiotics (Li, et al., 2020). The identification of resistance to common antibiotics like ciprofloxacin, gentamicin, and amoxicillin poses serious public health issues because RTE salads exclude heating, which is essential for microbial inactivation. Foodborne pathogen isolates of *Staphylococcus* spp. and *E. coli* have a well-established mechanism. Beyond complicating treatment, these resistant strains in food may also facilitate the transfer of resistance genes between harmless and pathogenic bacteria within the human gut microbiome. Additionally, *Klebsiella quasipneumoniae* and *E. coli*, which were both found in this study, are categorized as coliform bacteria. Coliforms are "indicator organisms," and if they are detected in huge quantities rather than in a single genus or species, it may indicate the lack of sanitary practices in food production.

S1 (Greek salad) contained a range of bacterial strains, some of which were resistant to gentamicin and amoxicillin while others were still susceptible to ciprofloxacin. S2 (apple salad) showed complete susceptibility to every antibiotic that was tested, suggesting that it was a premium product that was kept under appropriate microbiological control. Each of the three antibiotics showed distinct resistance profiles among the bacterial isolates in S3 (potato salad), indicating heterogeneity within the bacterial population. Amoxicillin-resistant bacteria were present in S5 (chicken, potatoes, and pineapple salad), which had the highest microbial diversity. This was probably caused by inappropriate handling or insufficient preservation of the components. The fact that this sample contained both *Staphylococcus* spp. and *E. coli* emphasizes how crucial it is to follow stringent cleanliness guidelines. *Staphylococcus* spp. and *E. coli* bacteria species were identified in S1, S3 and S5 samples.

PCR tests revealed no amplification for *E. coli* or *Staphylococcus* species in S4 (fresh mix salads), indicating the presence of other, potentially less dangerous, or unidentified bacteria. Sanger sequencing was used to address this, and the results showed the unexpected presence of the Gram-negative, possibly antibiotic-resistant bacterium *Klebsiella quasipneumoniae*. The microbial communities in ready-to-eat (RTE) salads can be varied and misleading, so this finding highlights the limits of depending only on species-specific PCR. The study underscores the importance of using advanced molecular tools like 16S rRNA gene sequencing for comprehensive detection of bacterial contaminants in food safety assessments.

Using the universal primer pair 27F and 1492R, 16S rRNA gene sequence from isolate S4-A was amplified, and Sanger sequencing was used to confirm the results. To verify its identity, the resulting sequence was manually aligned using MEGA and then analyzed using the BLASTn tool against the NCBI nucleotide database. Species-level matches were defined as those sequences having ≥98% identity. With accession number PV668983, the verified sequence was added to the NCBI GenBank database.

The study highlights how different types of salads have different patterns of microbial contamination and antibiotic resistance, which are influenced by things like ingredients, processing, and storage. Given that

resistant bacteria have been discovered in seemingly fresh products, it highlights the danger of antimicrobial resistance (AMR) spreading across the food chain.

Despite its value, this study was limited by its small sample size, sampling area and the use of only three antibiotics, which would not accurately reflect the range of resistance found in RTE salads. Seasonal changes, the potential of cross-contamination during retail handling, and the effect of packaging materials on bacterial growth should all be investigated in future studies. In the study by Abakari, et al., 2018, MacConkey agar, Salmonella-Shigella agar, Simmons Citrate Agar, and Mannitol Egg Yolk Polymyxin (MYP) agar were used for culturing. In contrast, Luria-Bertani (LB) medium, a versatile bacterial culture medium, was utilized in the present research study. The existence of bacteria resistant to antibiotics in these foods raises significant public health issues, highlighting the necessity of better hygienic procedures, frequent microbiological testing, consumer education campaigns, and more stringent laws governing the use of antibiotics in food processing and agriculture.

Conclusion

According to this study, there are significant health hazards associated with antibiotic resistant pathogenic bacteria, such as strains of *Staphylococcus, E. coli,* and *Klebsiella quasipneumoniae* found in ready-to-eat (RTE) salads sold in Sri Lankan supermarkets. Bacteria exhibited the highest resistance to amoxicillin, whereas ciprofloxacin was typically effective across samples. Apple salad was safe to eat because it didn't contain any antibiotic-resistant bacteria. Pathogenic, antibiotic-resistant bacteria were present in Greek salad, potato salad, fresh mix salad, chicken and pineapple salad, making them unfit for human consumption. The results highlight the importance of maintaining strict hygiene practices throughout the food production and supply chain to reduce the risk of infection from RTE salads.

Acknowledgment

I express my gratitude to everyone who helped and advised me during this research, offering words of wisdom and encouragement. I am grateful to my colleagues for their cooperation and motivation, and to my family for their unwavering support, which greatly contributed to the successful completion of this study.

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