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Identification of Antibiotic-Resistant Bacteria in Processed Meat Products Available in Local Markets from Five Selected Localities in Sri Lanka

U. Jayakody*[1], D. Vithanage[2]

[1]*Department of Applied Sciences, Faculty of Humanities and Sciences, Sri Lanka Institute of Information Technology, SLIIT-Malabe Campus, Malabe, 10115, Sri Lanka.*
[2] *Genetech Molecular Diagnostics and School of Gene Technology, 54, Kitulwatta Rd, Colombo, 00800, Sri Lanka.*

Corresponding author*: sahani.upadya@gmail.com

Abstract

This study focused on identifying antibiotic-resistant bacteria in processed meat products available in Sri Lankan local markets, considering the potential risks caused by inappropriate packaging and storage conditions. Five processed meat samples were purchased from five localities in Sri Lanka and examined on Luria-Bertani (LB) agar medium using both homogenised and direct culture techniques. The Kirby-Bauer disc diffusion method was used in the Antibiotic Sensitivity Test (ABST) to determine how bacteria responded to various antibiotics. Samples that were improperly packaged revealed the presence of antibiotic-resistant bacterial strains, exhibiting resistance to both ampicillin and amoxicillin, while ciprofloxacin sensitivity was observed in every tested bacterium. DNA was extracted from the antibiotic-resistant bacteria. *Escherichia coli* and *Staphylococcus* sp. were confirmed using Polymerase Chain Reaction (PCR) and agarose gel electrophoresis. Although PCR identified many isolates, it was unable to confirm two bacterial species; after additional DNA sequencing analysis, these two unidentified organisms were determined as *Enterobacter sp.* and *Psychrobacter piechaudii*. These results demonstrate the significance of appropriate packaging in avoiding the foodborne transmission of bacteria that are resistant to antibiotics. The study additionally indicates that to improve food safety and decrease antibiotic overuse, public awareness and stronger regulations are required. This study improves the understanding of how antibiotic resistance can spread through regularly consumed food products, which helps protect public health.

Keywords: Processed meat, *Escherichia coli.*, *Enterobacter* sp., *Psychrobacter piechaudii.*, *Staphylococcus* sp.

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Introduction

Processed meat consumption has consistently increased globally because of its low price, simplicity of use, and long shelf life (Shaltout, 2022). Because of the potential for antibiotic-resistant bacteria, the increased consumption of processed meat has raised concerns regarding public health (Bhutia, et al., 2021). The effectiveness of antibiotics in treating infections caused by bacteria is severely restricted by antibiotic resistance, which has become a major problem for global health. Treating infections becomes more difficult when antibiotic-resistant bacteria can resist treatment. Higher death rates, longer periods of recovery, and greater medical costs result from this (Capita & Calleja, 2013). Antibiotics can be used therapeutically non-therapeutically in food animals. The continued use of antibiotics in animal agriculture is the primary cause of the high incidence of bacteria resistant to antibiotics in food animals. When found in food products like meat, these resistant bacteria can transmit resistance throughout populations and raise the risk to public health. Moreover, they can spread resistance to other bacteria in the food chain. Agarose gel electrophoresis and polymerase chain reaction (PCR) have been utilised to increase the accuracy of antibiotic resistance bacterial detection (Bhutia, et al., 2021). The Sanger sequencing method was used to determine unidentified organisms. Sanger sequencing, also known as the dideoxy technique or chain termination, uses ddNTPs to determine the base order of DNA and end strand growth. Because of its high level of accuracy and long read capacity, it is recognised as the gold standard (Tamang, 2024). The processed meat products found in local markets in five selected districts of Sri Lanka are the focus of this study. Investigating the existence of bacteria resistant to the widely used antibiotics amoxicillin, ampicillin, and ciprofloxacin, identifying the bacterial species involved, and evaluating their spread and any negative health effects are the main goals. The efficiency of various culturing methods, including direct and homogenised methods, in isolating bacterial colonies is compared in this study. It is expected that the results of this study will significantly improve the food safety laws and regulations in Sri Lanka. The objectives of this research were to find antibiotic-resistant bacteria in locally accessible processed meat products, evaluate the potential dangers to consumers, compare the effectiveness of direct and homogenised culturing techniques, and assess the impact of improper packaging on bacterial contamination levels.

Materials and Methods

Five samples of processed chicken meat were purchased from 5 local markets in the districts of Colombo, Gampaha, Kalutara, Kurunegala, and Puttalam in Sri Lanka. To represent handling procedures in the real world, samples were collected in the packing conditions in which they were purchased. Both non-vacuum-sealed and vacuum-sealed samples were used to determine how packaging techniques affected the bacterial contamination. Two of the five processed meat samples were correctly vacuum-sealed and kept in controlled conditions, while the other three were improperly packed without vacuum sealing. All the samples were double-wrapped in UV-sterilised ziplock bags, blindly coded, and stored at 4°C for 24 hours (Attien, et al., 2013). They were transported on ice in aseptic conditions (Shaltout, 2022). Samples were prepared for both the direct and homogenised culturing methods separately (Gebre, 2012). Samples of processed meat were cultured on Luria-Bertani (LB) media. Both direct and homogenised culturing techniques were used. Direct samples were streak-plated, while homogenised samples were spread-plated. Bacterial colonies were identified by their morphological characteristics after a 24-hour incubation period at 37°C (Fayemi, et al., 2021). The antibiotic susceptibility of bacterial isolates was evaluated using the Kirby-Bauer disc diffusion method and three antibiotics: ampicillin, ciprofloxacin, and amoxicillin. The zones of inhibition were interpreted based on the Clinical Laboratory Standards Institute (CLSI) guidelines to classify bacterial isolates as resistant (R), susceptible (S), or intermediate (I) (Gebre, 2012). Gram-staining is performed on bacteria that are resistant to tested antibiotics. The boiling method was used to extract

DNA from bacterial colonies that were resistant to antibiotics. Bacterial DNA was confirmed by universal bacterial PCR (Samani, et al., 2021). PCR was performed using species-specific primers that target *Staphylococcus* species (Martineau, et al., 2001) and *Escherichia coli* (Spano et al.,2005), followed by agarose gel electrophoresis to identify the bacterial species. Sanger sequencing analysis was conducted to identify unknown bacterial organisms (Tamang, 2024). Bacterial species were determined using the National Center for Biotechnology Information (NCBI) BLAST after obtaining the Sanger sequencing results.

Results

Antibiotic Sensitivity Test Results

Antibiotic-resistant bacteria were discovered in non-vacuum-sealed samples (Figure 1), showing resistance to ampicillin and amoxicillin, while all bacterial strains remained susceptible to ciprofloxacin (Table 1).

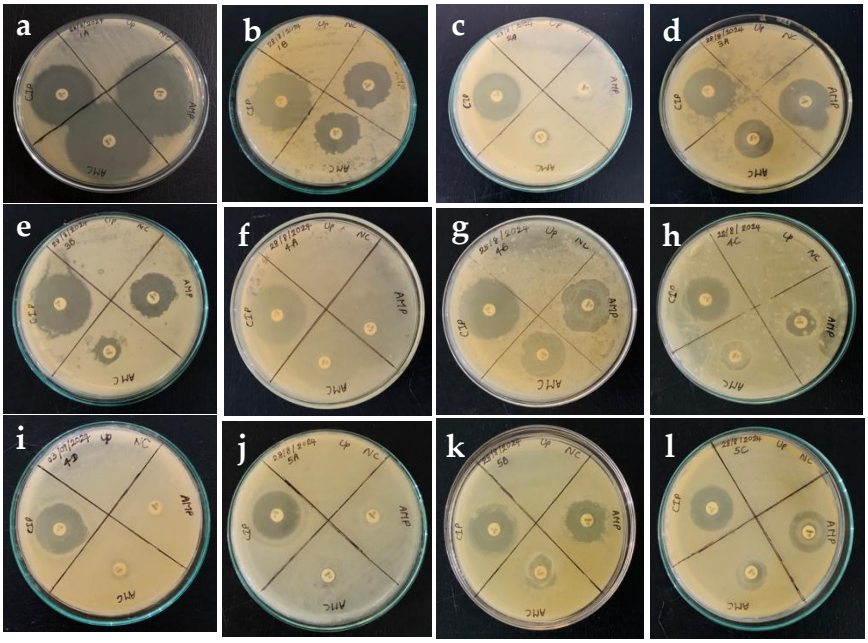


Figure 1: *Antibiotic Sensitivity Test Results of sub-samples from all five processed meat samples.*

a).1-A, b).1-B, c).2-A, d).3-A, e).3-B, f).4-A, g).4-B, h).4-C, i).4-D, j).5-A, k).5-B, l).5-C

PCR and Agarose Gel Electrophoresis

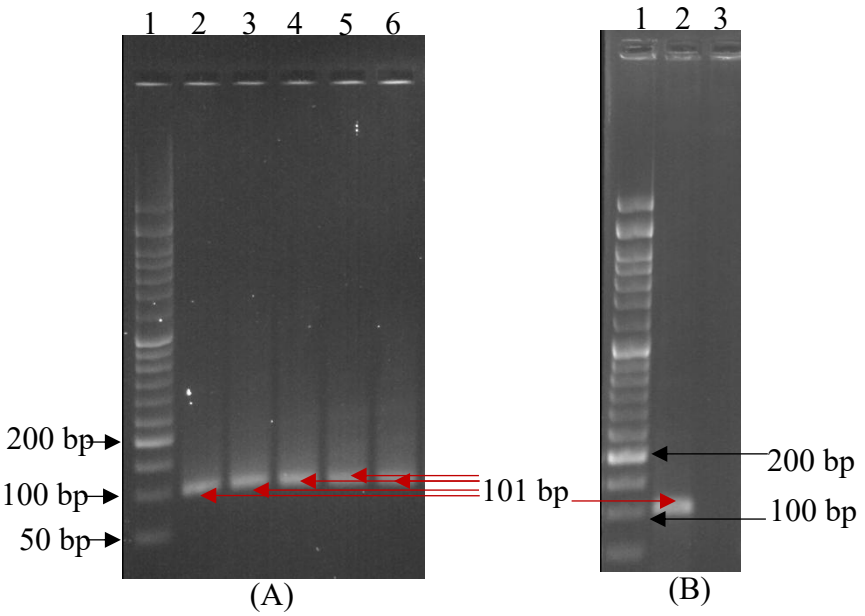
Universal bacterial PCR confirmed the presence of bacterial colonies with DNA bands at 371 bp in all examined samples and the positive control. According to Gram-staining results, all gram-negative rods in tested samples that were resistant to antibiotics were confirmed as *Escherichia coli* from the PCR analysis done with *Escherichia coli-specific* primers (Figure 2). Using *Staphylococcus* species-specific primers, the PCR confirmed that the gram-positive cocci in clusters were *Staphylococcus* species (Figure 3).

Table 1: *The Results Summary of gram staining, antibiotic Resistance and Identified Antibiotic-Resistant Bacteria.*

Sample No.	Vacuum Status	Sub-Sample Number	Resistant Antibiotic Groups			Identified Antibiotic-Resistant Bacteria
			Amoxicillin	Ampicillin	Ciprofloxacin	
1	Vacuum sealed	Gram-Negative Rods	Sensitive	Sensitive	Sensitive	_
		Gram-Positive bacillus	Sensitive	Sensitive	Sensitive	
2	Non – vacuum sealed	Gram-Negative Rods	Resistant	Resistant	Sensitive	• *Escherichia coli*
		Gram-Positive bacillus	Sensitive	Sensitive	Sensitive	_
3	Vacuum sealed	Gram-Positive bacillus	Intermediate	Sensitive	Sensitive	_
		Gram-Negative bacillus	Sensitive	Sensitive	Sensitive	
		Gram-Positive cocci in clusters	Sensitive	Sensitive	Sensitive	
4	Non – vacuum sealed	Gram-Negative Rods	Resistant	Resistant	Sensitive	• *Escherichia coli* • *Enterobacter sp.*
		Gram-Negative bacillus	Resistant	Resistant	Sensitive	
		Gram-negative coccobacillus	Resistant	Sensitive	Sensitive	
		Gram-negative Rods	Resistant	Resistant	Sensitive	
5	Non – vacuum sealed	Gram-negative Rods	Resistant	Resistant	Sensitive	• *Escherichia coli* • *Staphylococcus sp.* • *Psychrobacter piechaudii*
		Gram-positive cocci in clusters	Resistant	Sensitive	Sensitive	
		Gram-negative coccobacillus	Resistant	Sensitive	Sensitive	

Psychrobacter piechaudii strain SC-YG 1A was determined using partial 16S rRNA gene sequencing (929 bp). The sequence was submitted to GenBank under the accession number PV668968. *Enterobacter sp.* strain DS'3

was determined using partial 16S rRNA gene sequencing (1009 bp). The sequence was submitted to GenBank under the accession number PV668959.



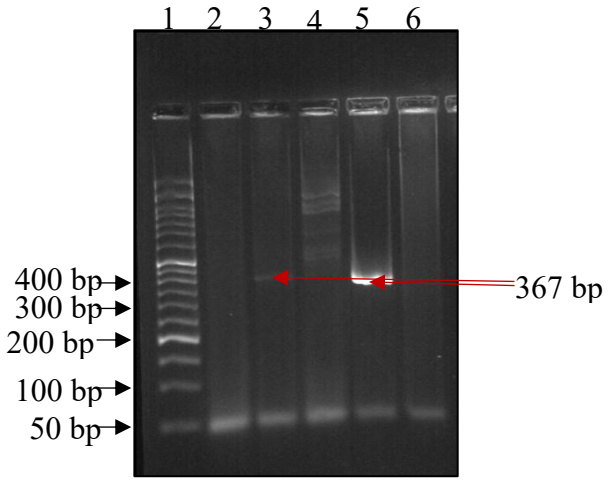
(A)

Lane	Sample
1	50 bp Ladder
2	2-A
3	3-B
4	4-A
5	4-B
6	5-A

(B)

Lane	Sample
1	50 bp Ladder
2	Positive Control
3	Negative Control

Figure 2: *Agarose gel electrophoresis images of Escherichia coli PCR products. (A) & (B). DNA bands at 101 bp are confirmed the presence of Escherichia coli in all the examined samples and the positive control.*



Lane	Sample
1	50 bp Ladder
2	4-C
3	5-B
4	5-C
5	Positive Control
6	Negative Control

Figure 3: *Agarose gel electrophoresis image of Staphylococcus sp. PCR products. DNA bands at 367 bp are confirmed the presence of Staphylococcus sp. in the two examined samples and the positive control.*

Discussion

Identifying the bacteria that are resistant to antibiotics in processed meat products available in Sri Lankan local markets was the primary objective of this research study. Only non-vacuum-sealed samples included antibiotic-resistant bacteria. The study discovered coliform bacteria, such as *Escherichia coli* and *Enterobacter sp.* Their existence does not always mean that there are pathogens present, but it does indicate poor hygiene conditions. Antibiotic susceptibility test indicated that bacterial isolates from non-vacuum-sealed samples were resistant to amoxicillin and ampicillin, while all examined isolates were sensitive to ciprofloxacin. One bacterial colony in sample three displayed an intermediate inhibitory zone, which was by heteroresistance. When a bacterial population contains sub-populations with both sensitive and less sensitive cells, an intermediate zone is created. It has been discovered that the homogenised culturing approach produces more successful bacterial colony growth than direct culturing. PCR and agarose gel electrophoresis were utilised in this study to confirm the detection of *Staphylococcus* sp. and *Escherichia coli*. High specificity and sensitivity are offered by molecular techniques, which are now crucial tools for modern microbiological diagnostics (Alvarado, et al., 2017). *Psychrobacter piechaudii* and *Enterobacter sp.* were determined by sequencing analysis. Bacterial isolates are frequently identified through Sanger sequencing of the 16S rRNA gene. Sanger sequencing is perfect for confirming results from other techniques because of its great accuracy. This method is straightforward, reproducible outcomes and well-established. Long read lengths and high-quality, easily interpretable data are provided by the method, which is appropriate for small-scale investigations and short DNA regions (Tamang, 2024). The NCBI BLAST tool was used to analyse the 16S rRNA gene sequence, which confirmed the identity of the bacterial isolate

The small sample size of this study is one of its limitations, which could limit the extent to which these findings can be used. Future research with more samples could verify and expand these findings. Additionally, focussing on just three antibiotics provides an incomplete understanding of the wider range of resistance patterns that may exist. A more complete resistance profile would be provided by future studies with more kinds of antibiotics. *Salmonella* was identified in previous research utilising the traditional culture method, ISO 6579 (2002). The McFarland turbidity standard was used for creating the *salmonella* culture suspension. Mueller Hinton agar medium was used (Gebre, 2012). As a versatile bacterial culture medium, Luria-Bertani culture medium was utilised in this research study. It supports the growth of a variety of facultative microorganisms. DNA was directly extracted from samples of processed meat in a previous study (Alvarado, et al., 2017). However, the boiling method was used in this investigation to extract DNA from grown bacterial cultures. Shiga toxigenic *Escherichia coli* (STEC), *Salmonella* (Gebre, 2012), *Enterococcus faecalis*, and antibiotic-resistant lactic acid bacteria (Aquilanti, et al., 2007) have been found in processed meat in previous studies. This study found that antibiotic-resistant *Staphylococcus sp*., *Escherichia coli, Psychrobacter piechaudii*, and *Enterobacter sp.* are present in processed meats.

Conclusion

The research confirmed that bacteria resistant to ampicillin as a specifically *Escherichia coli, Staphylococcus species, Enterobacter sp.*, and *Psychrobacter piechaudii*, were present in processed meat products from Sri Lankan markets. The most important observation was that vacuum-sealed samples did not exhibit antibiotic resistance, while non-vacuum-sealed samples had a greater frequency of contamination. This highlights the importance of appropriate storage and packaging procedures to stop bacterial contamination and protect the safety of the public. Since all bacterial strains were susceptible to ciprofloxacin, it was determined that this antibiotic was the most effective of those tested. These results emphasise the significance of strictly following food safety laws, especially in the preparation and packing of meat

products. This study advances the understanding of antibiotic resistance in food items and follows actions that protect the health and welfare of consumers.

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