



Faculty of Resource Science and Technology

**Microbiological Quality and Safety Assessment of Selected Food Premises
in Sarawak**

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Microbiological Quality and Safety Assessment of Selected Food Premises in
Sarawak

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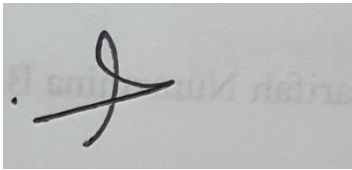
Faculty of Resource Science and Technology

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.



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ABSTRACT

Foodborne pathogens on food-contact surfaces pose a significant public health concern, particularly in food service establishments, where inadequate sanitation can facilitate cross-contamination and the transmission of antimicrobial-resistant bacteria. In this study, the microbiological quality of food-contact surfaces in designated P2 category food premises in Kuching and Kota Samarahan, Sarawak, was examined, with a focus on the prevalence of contamination, antimicrobial resistance profiles, and strategies for food safety management. The most probable number–polymerase chain reaction (MPN-PCR) method was employed to analyse a total of 150 samples from spoons, forks, plates, tables, and glasses. Subsequently, 16S rRNA sequencing was conducted to identify the species and conduct antibiotic susceptibility testing. The results indicated that 46% of the samples were contaminated, with microbial concentrations ranging from 3 MPN/mL to over 1,100 MPN/ml. The genus *Enterobacter* spp. was the most prevalent, and 34.78% of the isolates exhibited multidrug resistance, which included resistance to carbapenems. Statistical analyses verified that there was a substantial variation in bacterial concentrations among outlets, with Kota Samarahan exhibiting a higher degree of variability than Kuching. The hazard analysis revealed six critical control points, which are primarily associated with the handling of fresh meat, refrigerated storage, cooking, and reheating. Only two outlets in Kuching had documented Hazard Analysis and Critical Control Point (HACCP) plans, while none were observed in Kota Samarahan, despite the ubiquitous adoption of baseline food safety programs such as Clean, Safe, and Healthy (BeSS) and Food Safety is the Responsibility of the Industry (MeSTI). These findings suggest that sanitation practices are systemically flawed and that HACCP implementation is inadequate. The study concludes that food-contact surfaces in Sarawak food establishments act as reservoirs for antimicrobial-

resistant bacteria, highlighting the necessity of implementing more stringent sanitation practices, conducting routine microbial monitoring, and promoting the broader adoption of HACCP to protect public health.

Keywords: Food contact surfaces, foodborne bacteria, P2 category food establishment, multi-drug resistance, HACCP implementation

Penilaian Kualiti dan Keselamatan Mikrobiologi di Premis Makanan Terpilih di Sarawak

ABSTRAK

Patogen bawaan makanan pada permukaan sentuhan makanan menimbulkan kebimbangan kesihatan awam yang ketara, terutamanya dalam kalangan kedai perkhidmatan makanan, di mana sanitasi yang tidak mencukupi boleh memudahkan pencemaran silang dan penghantaran bakteria tahan antimikrob. Dalam kajian ini, kualiti mikrobiologi permukaan sentuhan makanan di premis makanan kategori P2 yang ditetapkan di Kuching dan Kota Samarahan, Sarawak, telah diperiksa, dengan fokus pada kelaziman pencemaran, profil rintangan antimikrob, dan strategi untuk pengurusan keselamatan makanan. Kaedah tindak balas rantai nombor-polimerase (MPN-PCR) yang paling berkemungkinan telah digunakan untuk menganalisis sejumlah 150 sampel daripada sudu, garpu, pinggan, meja dan gelas. Selepas itu, penjujukan rRNA 16S telah dijalankan untuk mengenal pasti spesies dan menjalankan ujian kerentanan antibiotik. Keputusan menunjukkan bahawa 46% daripada sampel telah tercemar, dengan kepekatan mikrob antara 3 MPN/mL hingga lebih 1,100 MPN/mL. Genus Enterobacter spp. adalah yang paling lazim, dan 34.78% daripada isolat mempamerkan rintangan multidrug, yang termasuk rintangan kepada carbapenem. Analisis statistik mengesahkan bahawa terdapat variasi besar dalam kepekatan bakteria di kalangan kedai, dengan Kota Samarahan mempamerkan tahap kebolehubahan yang lebih tinggi daripada Kuching. Analisis bahaya mendedahkan enam titik kawalan kritikal, yang dikaitkan terutamanya dengan pengendalian daging segar, penyimpanan dalam peti sejuk, memasak dan memanaskan semula. Hanya dua cawangan di Kuching telah mendokumentasikan rancangan Analisis Bahaya dan Titik Kawalan Kritikal (HACCP), manakala tiada satu pun diperhatikan di Kota Samarahan, walaupun terdapat penggunaan

program keselamatan makanan asas seperti MeSTI (Makanan Selamat Tanggungjawab Industri) dan BeSS (Bersih, Selamat dan Sihat). Penemuan ini menunjukkan bahawa amalan sanitasi adalah cacat secara sistemik dan pelaksanaan HACCP tidak mencukupi. Kajian itu menyimpulkan bahawa permukaan sentuhan makanan di kedai perkhidmatan makanan di Sarawak bertindak sebagai takungan bakteria tahan antimikrob, menonjolkan keperluan untuk melaksanakan amalan sanitasi yang lebih ketat, menjalankan pemantauan mikrob rutin, dan menggalakkan penggunaan HACCP yang lebih meluas untuk melindungi kesihatan awam.

Kata kunci: *Permukaan sentuhan makanan, bakteria bawaan makanan, premis makanan kategori P2, rintangan pelbagai ubat, pelaksanaan HACCP*

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LIST OF ABBREVIATIONS

AMR	Antimicrobial resistance
BeSS	Clean, Safe, and Healthy (“Bersih, Selamat dan Sihat”)
CCPs	Critical Control Points
CLSI	Clinical and Laboratory Standards Institute
FBD	Food borne diseases
FDA	Food and Drug Administration
FoSIM	Food Safety Information System of Malaysia
GMP	Good Manufacturing Practices
HACCP	Hazard Analysis and Critical Control Points
KAP	Knowledge, Attitude and Practice
MAR	Multiple Antibiotic Resistance
MeSTI	Food Safety is the Responsibility of the Industry (“Makanan Selamat Tanggungjawab Industri”)
MOH	Ministry of Health Malaysia
MPN	Most Probable Number
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Study Background

Foodborne diseases are a substantial global public health burden, with the World Health Organization (WHO) estimating that unsafe food causes approximately 600 million illnesses and 420,000 fatalities annually (WHO, 2024). Contaminated food-contact surfaces are widely acknowledged as critical vehicles for the transmission of microorganisms. In food service establishments, the primary points of contact between food, handlers, and consumers are prepared tables and commonly used utensils, including spoons, forks, plates, and glasses. If the cleansing and disinfection procedures are inadequate, these surfaces can act as reservoirs of pathogens and promote cross-contamination. *Escherichia coli*, *Bacillus cereus*, and coliforms have been consistently recorded on utensils and ready-to-eat foods in restaurant and bazaar contexts in Malaysia (Nahar & Mahyudin, 2018; Azmi et al., 2023; Mohamad et al., 2024). Comparable research conducted in other regions, such as Nigeria, Cambodia, and Thailand, reported contamination rates on cutting boards and implements ranging from 40% to 60% (Schwan et al., 2020; Tasanapak et al., 2023; Umaru, 2023). This research emphasizes the significance of assessing the prevalence of microbial contamination on food-contact surfaces, particularly in Sarawak, where empirical data is scarce, and highlights systemic hygiene deficiencies in food service environments.

In addition to the issue of surface contamination, antimicrobial resistance (AMR) has emerged as a significant threat to public health and food safety. AMR is one of the ten most significant global health concerns of the 21st century (Oliveira et al., 2024). Resistance

bacteria may endure in food service environments, enduring inadequate cleaning procedures, and subsequently disseminating to consumers through cross-contamination. *Enterobacter*, *Klebsiella*, and *Staphylococcus* are pathogens that have been reported to be resistant to multiple antibiotics, including last-line carbapenems, on an international scale (Cosby et al., 2008; Ohman et al., 2023). These pathogens are of particular concern as the threat of treatment failures is increasing and therapeutic options for infected individuals are restricted by the introduction of such multidrug-resistant organisms into the food chain. Malaysia has national surveillance programs for AMR in clinical and agricultural contexts (Mariappan et al., 2021); however, there is a lack of research that has examined the resistance patterns of pathogenic bacteria isolated from food-contact surfaces in food premises. Thus, it is imperative to comprehend the resistance characteristics of these pathogens in order to evaluate risk and direct antimicrobial stewardship strategies in the food industry.

Food safety management systems (FSMS) are crucial for safeguarding consumers and reducing the risk of contamination. In order to encourage adherence to the minimum hygiene standards, Malaysia has implemented several baseline programs, including Clean, Safe, and Healthy (BeSS) and Food Safety is the Responsibility of the Industry (MeSTI). In spite of the fact that these programs establish a basis for safer food handling, they do not necessitate systematic hazard analysis or microbiological verification. On the other hand, the Hazard Analysis and Critical Control Point (HACCP) system, which is generally acknowledged as the international standard for preventive food safety management, prioritizes the identification, monitoring, and management of hazards during critical stages of food preparation (European Commission, 2005; Food and Drug Administration, 2023; Codex Alimentarius, 2023). Microbial hazards in food service and processing environments have been significantly diminished through the implementation of HACCP (Abdallah-Ruiz

et al., 2022; Zaki et al., 2024). The usage of HACCP, though, varies throughout Malaysia. Smaller food businesses that come under the P2 category and use MeSTI and BeSS may find it tough to apply structured preventative controls over operations. Halim et al. (2024) state that this lack of planning makes HACCP necessary for bigger companies whose primary goal is exporting. This disorganized implementation makes it harder to do so.

The ongoing presence of microbial contamination on surfaces that come into contact with food, the growing risk of antimicrobial resistance, and the inconsistent use of comprehensive food safety management systems all show how important it is to have integrated assessments in local food service settings. Surface contamination does not provide enough risk clarification since it does not consider the microorganisms' resistance qualities or the effectiveness of management techniques aimed at controlling them. Therefore, it is vital to assess both dimensions together. These evaluations are critical in places like Sarawak, where smaller businesses and real-world data are more common in the food service industry. In addition to filling in gaps in the knowledge, gathering localised evidence on microbial prevalence, resistance profiles, and the implementation of food safety management systems gives policymakers important information that can help them make better decisions, improve regulatory oversight, and create targeted interventions to protect public health.

1.2 Problem Statement

Research conducted by Chen et al. (2021) and Wahab et al. (2025), among others, in Malaysia have shown that food contact surfaces are a significant reservoir for infectious diseases that have not been thoroughly studied (Zulfakar et al., 2018; Nahar & Mahyudin, 2018; Sabaruddin et al., 2019; Wahab et al., 2025). Few studies have meticulously assessed

the frequency and species composition of bacteria on surfaces that come into contact with food in commercial food establishments throughout several states in Malaysia. Despite reports from institutional and boarding school outbreaks and national surveillance indicating that contaminated food and lack of sanitation are leading causes of food poisoning in Malaysia, this remains the case (Bujang et al., 2023). In Malaysia, studies have detected *Listeria* spp. and other pathogens on direct and indirect food contact surfaces, indicating that surfaces used in preparation and service can carry clinically relevant organisms (Chen et al., 2021). Concerning findings have emerged from the surface contamination study thus far. This is especially important in Sarawak, where studies in certain areas, such as Padawan, Kuching South, and North, have found possible breeding grounds for food-borne diseases, such as overcrowded marketplaces and settings where street vendors sell their wares (Mohamad et al., 2024). However, systematic surface-sampling data across different surfaces and buildings is scarce when evaluating regional risk patterns and prevalence. Without accurate prevalence estimates for surfaces (by surface type, premise type, and location), public health authorities and premises managers cannot prioritize cleaning regimens, supplies, or training where it is most needed.

The resistance profiles of isolates from food premises in Malaysia are not yet sufficiently characterized, despite the fact that antibiotic resistance among bacteria isolated from food and environmental surfaces is an emerging threat (Cave et al., 2021). Global and regional meta-analyses indicate that the prevalence of AMR in foodborne pathogens is on the rise (Tao et al., 2022). In Malaysia, research has identified multi-resistant organisms in food-related environments, including food products, animal sources, and environmental reservoirs (Hartantyo et al., 2020; Cheesman et al., 2023). Nevertheless, there are only a handful of studies in Malaysia that combine surface sampling from food premises with

comprehensive multi-antibiotic susceptibility profiling to determine multiple antibiotic resistance (MAR) indices or identify resistance genes. In contrast, numerous studies have examined other food sources, such as raw meats, fish, and aquaculture (Yap et al., 2022; Elexson et al., 2023; Natrah et al., 2025). This results in a gap: contaminated surfaces can act as both vectors for the transmission of resistant strains to consumers and food handlers and as pathogen reservoirs (Oliveira et al., 2024). Comparative assessments indicate that the prevalence of AMR in food matrices in Malaysia is in line with global upward trends (Naeemmudeen et al., 2021). However, there is a scarcity of direct evidence regarding surface-associated AMR, particularly in Sarawak. Therefore, the documentation of MAR profiles from surface isolates will address a critical knowledge vacuum and help inform both clinical and food safety responses. A baseline MAR profile is essential for risk assessment and antibiotic stewardship planning, as routine contaminated infections may become more difficult to treat and control if surfaces harbour multi-drug resistant bacteria.

The implementation and conformance with HACCP and related food safety management systems in Malaysian food premises are inconsistent, and weaknesses in HACCP application could result in critical control gaps that permit the persistence of surface contamination and AMR transmission. Food safety in Malaysia encompasses regulatory frameworks, HACCP guidance, and inspection programs. However, numerous studies of Small and Medium Enterprises (SMEs), street vendors, and local bazaars have identified obstacles to the proper implementation of HACCP, including inconsistent documentation, resource constraints, limited awareness, and inadequate training (Dima et al., 2024). Implementation studies have shown that the mere presence of HACCP documentation does not guarantee effective control unless critical control points (CCPs) are correctly identified and monitored, despite the fact that HACCP remains the leading preventive framework on a

global scale (Lai et al., 2024). This is a particular concern when the cleaning and monitoring of contact surfaces are neglected. In Sarawak, surveys of vendor hygiene and targeted food quality studies for example, at bazaars, indicate that operational practices are highly variable (Azmi et al., 2023; Azmi et al., 2025). To establish a connection between management systems and microbiological outcomes, such as surface prevalence and AMR profiles, a formal, on-site HACCP evaluation (observational auditing plus microbiological verification) is required (Mizanur et al., 2012; Jores et al., 2018). It is possible to identify operational gaps such as cleaning frequency, disinfectant effectiveness, critical control point monitoring that directly influence surface contamination and downstream infection/AMR risk by evaluating HACCP fidelity, rather than merely adoption.

Together, these three issues establish a comprehensive framework for evaluating food safety concerns, as they are intrinsically interdependent. The baseline level of microbial hazard present on food contact surfaces is established by understanding contamination prevalence, which emphasizes the extent to which these surfaces may contribute to the transmission of infectious diseases. Nevertheless, the mere existence of microorganisms does not provide a comprehensive indication of the potential public health consequences. Consequently, the characterization of their antibiotic resistance profiles is essential for determining the severity of the threat, as multi-drug resistant strains pose a greater risk by restricting treatment options and enhancing the probability of protracted or severe outbreaks. Lastly, the study is able to determine whether the current preventive measures are effectively mitigating these risks or whether operational gaps, such as inadequate cleaning protocols, poor monitoring of critical control points, or a lack of staff training, are contributing to both bacterial persistence and resistance development by evaluating the implementation of HACCP systems. The research establishes a logical sequence from hazard identification to

risk assessment and control by linking these three components, which not only quantifies contamination and resistance but also investigates the underlying management practices that influence these outcomes.

A number of innovative contributions are presented in this study to resolve these issues. It is one of the first comprehensive studies to examine the prevalence of microbiological contamination on food contact surfaces in P2 enterprises in Sarawak, and it provides new baseline data that was not available before. Secondly, it assesses bacterial isolates from these surfaces using antimicrobial resistance profiling, which gives the first empirical proof of resistance patterns and multiple antibiotic resistance (MAR) indices in this practical setting. Finally, the study uses a seldom-used technique in Malaysia which is directly linking compliance status to microbiological and resistance outcomes to evaluate the HACCP implementation in P2 establishments. The study finds that by incorporating contamination assessment, antimicrobial resistance profiling, and HACCP evaluation into a cohesive multidimensional framework, food safety issues and management strategies can be comprehensively addressed. These unique aspects set the study apart from earlier, more disjointed efforts and ensured that its results added to scientific understanding and strengthened evidence-based food safety regulations in Sarawak.

This study was motivated by three primary hypotheses designed to clarify the potential hazards associated with food safety in P2 category food establishments. The initial hypothesis posited that the incidence of pathogenic bacteria varies considerably across various food contact surfaces, suggesting that certain surfaces may be more susceptible to contamination than others. Subsequently, it was proposed that contaminated bacterial isolates were resistant to numerous antibiotics frequently employed in these regions. This

underscores the potential threat to public health that antimicrobial-resistant bacteria in the food supply chain may pose. The final hypothesis was that there was a significant correlation between the quantity of microorganisms present and the extent to which HACCP was adhered to. This implies that even in certified establishments, improved food safety and hygiene may result from more stringent adherence to HACCP regulations.

1.3 Objectives

The study aimed to assess the microbiological quality of specific food establishments and the execution of HACCP protocols within the Sarawak food service sector. This can be accomplished by the subsequent objectives:

- i. To determine the prevalence of foodborne bacteria on different food contact surfaces from selected food premises
- ii. To investigate the MAR profile of the positive samples
- iii. To evaluate the HACCP implementation in the selected food premises

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to Food Safety

Malaysia places a high priority on food safety as a public health and economic issue because bacterial hazards like *Salmonella* spp., pathogenic *Escherichia coli*, *Staphylococcus aureus*, *Vibrio* spp., and *Bacillus cereus* are always present in the food chain (Cheesman et al., 2023). Recent research and evaluations have shown that metagenomics is increasingly used with culture-based approaches to find infections and resistance genes in Malaysian produce and meats. This signifies a national movement towards One Health surveillance (Quek et al., 2025). This method is part of Malaysia's current AMR Action Plan for 2022 to 2026, which includes people, animals, and the environment (Chung, 2025). The Food Act 1983, the Food Regulations 1985, and the Food Hygiene Regulations 2009 are the three main laws that make up Malaysia's food safety system (Halim et al., 2024). These frameworks give the Ministry of Health (MOH) the power to do inspections and enforcement and set legislative standards for food safety, hygiene, and training for food handlers. Recent changes show that these rules are still changing. In February 2025, the Ministry of Health (MOH) issued draft changes to the Food Regulations 1985. These changes included new rules for ensuring imported food is safe, stricter cleanliness standards, and new labelling rules (Food Compliance International, 2025). Malaysia also told the World Trade Organisation that imported food must come from facilities that follow recognised food safety assurance programs, like the Hazard Analysis and Critical Control Point (HACCP) system or Good Manufacturing Practices (GMP). The Ministry of Health (MOH) must check the supporting documents (Regulatory Representatives and Managers Association, 2025).

Additional analysis of national trends is provided by the surveillance of foodborne illnesses. In a study conducted by Hassan et al. (2023), the average national incidence rate of 561 food-poisoning cases per 100,000 population was reported. The study analysed data from 2004–2014 and found that climatic factors, such as temperature, substantially influenced the rate of cases. This follows a peak of 516 cases in 2019, a subsequent decline in 2020 and 2021, and a persistently high incidence in school settings, despite ongoing interventions by the MOH (Aspian et al, 2024). Nevertheless, Lee (2025) reported a 20% decrease in notifications from January to May 2025 in comparison to the same period in 2024, as well as a substantial increase in Clean and Safe Recognition (BeSS) certified premises. This suggests that hygiene compliance has improved in the short term.

Peninsular Malaysia has a more recent and extensive evidence base in the food sectors and urban contexts. Aziz et al. (2024) conducted a study in the Klang Valley that documented vendor knowledge-attitude-practice (KAP) gaps and determinants of safer practice, including stall type, income, and training. This study underscores the necessity of routine education and enforcement in high-density street-food environments. The concurrent detection of hazardous pathogens and AMR genes in Kinta Valley, Perak was demonstrated in complementary studies using shotgun metagenomics. This demonstrates methodological advancements that can improve risk prioritization in supply chains that serve Peninsular urban markets (Quek et al., 2025).

In contrast, research in Sarawak is more recent but more restricted, with a number of focused studies on Ramadan bazaar cuisines in Kuching since 2023–2025 (Azmi et al., 2023; Mohamad et al., 2024; Azmi et al., 2025). These studies report microbiological contamination in noodles and beverages, including the detection of *S. aureus*, *B. cereus*, and

indicators that exceed guideline levels (Mohamad et al., 2024; Azmi et al., 2025). Additionally, one study found that *B. cereus* isolates were resistant to common beta-lactams (Azmi et al., 2023). Previous KAP surveys conducted among Padawan and Kuching vendors also identified deficiencies in hygiene and training, despite the fact that these datasets are outdated and require updating (Jores et al., 2018). Even though these datasets are outdated and need updating, previous KAP surveys among vendors in Padawan and Kuching similarly found shortcomings in hygiene and training (Jores et al., 2018). Overall, the Sarawak evidence highlights the significance of small sellers and ready-to-eat bazaar items as inspection and training priority nodes. It also shows that there is a lack of research outside of Kuching. In HACCP-certified food establishments, there is less research on critical control points, hazard analysis, and surfaces that come into contact with food.

Even with these new ideas, there are still significant gaps in food safety research in Sarawak. An important problem is the insufficient systematic studies on food contact surfaces, like cutting boards, cutlery, and preparation tables, although, these surfaces are universally acknowledged as primary conduits for cross-contamination (Kirchner et al., 2021; Lai et al., 2024). Also, it is unclear how well HACCP systems work in the formal sector because there have not been any real-world data of HACCP-certified P2 category food businesses in Sarawak. The lack of data makes it hard to tell if the dangers of contamination are mostly linked to informal street vendors or if they also apply to institutional and commercial food environments.

2.1.1 Bridging the Evidence Gap: Comparative Analysis of Food Safety Studies in Peninsular Malaysia and Sarawak

In comparison, Peninsular Malaysia boasts greater resources, including more studies, methods, and cutting-edge technologies like metagenomics. This technology can identify microbial profiles and antibiotic resistance more accurately (Quek et al., 2025). As it enables more accurate risk assessments and tailored treatments, employing massive volumes of KAP data from urban regions can assist peninsular studies. Conversely, in Sarawak, recent research has looked chiefly at ready-to-eat foods associated with celebrations in Kuching bazaars (Jores et al., 2018; Azmi et al., 2025). Considering the small sample size and lack of published information, very little is known about the risks associated with institutional kitchens, indigenous food settings, and mass catering. Research by Sageng et al. (2024) based on ethnographic research on the cooking practices of the Bidayuh community highlight the importance of food safety advertising that respects cultural traditions, follows traditional preparation techniques, and adheres to community dining practices. This alternative to standard metropolitan approaches is crucial. In light of these results, it is evident that risk communication frameworks and monitoring tactics tailored to each state are necessary to guarantee the safety of regional foods while meeting federal requirements.

Peninsular Malaysia's food safety investigation encompasses a wide range of entities, including institutional facilities, street vendors, and HACCP-certified retail establishments. For example, examining the microbiological condition of surfaces that come into contact with food was the focus of one study in the Klang Valley. Nahar and Mahyudin (2018) conducted a study that demonstrated that inadequate cleansing procedures resulted in the contamination of restaurant utensils with *Escherichia coli*. Meanwhile, Zulfakar et al. (2018)

conducted research that indicated an increase in the prevalence of microorganisms on surfaces, particularly cutting boards, in university cafeterias. Prior research has resulted in the same conclusion. It is even more imperative to ensure that surfaces are sanitary in order to prevent the transmission of pathogens. Extensive sanitation monitoring is further emphasized by research on utensils and surfaces. This is consistent with the findings of Sabaruddin et al. (2019), who discovered that food courts contained *E. coli* and *Staphylococcus aureus*. This research demonstrates that risk assessments must encompass monitoring surfaces that come into contact with food to manage food safety effectively.

Two imperative research gaps are underscored in Sarawak by the absence of analogous investigations. Initially, there is a lack of systematic assessments of microbial contamination on food contact surfaces in both formal and informal environments. Secondly, the operational effectiveness and compliance of HACCP-certified premises in the state have not been assessed, resulting in an uncertainty regarding the performance of hazard control and verification systems. In order to rectify these deficiencies, it is feasible to incorporate molecular detection methodologies that are currently implemented in Peninsular Malaysia into methodologically rigorous, region-specific investigations. This would enhance the sensitivity of detection and foster a more profound comprehension of the dynamics of AMR. In summary, it is essential to strengthen Sarawak's research base to ensure the fair implementation of Malaysia's food safety policies. This will guarantee that the risk control standards are uniform across Peninsular and East Malaysia, while also allowing for the distinct operational landscapes and culinary cultures of each region.

2.2 Foodborne Diseases (FBDs): Prevalence and Public Health Impact

The global public health burden is still consequential due to FBDs. The World Health Organization (WHO) estimates that 600 million people get food poisoning every year, with 420,000 losing their lives. The number of affected children under five is even higher (WHO, 2024). These worldwide numbers show diverse causes (bacteria, viruses, parasites, poisons) and substantial regional and socioeconomic heterogeneity. Due to inadequate water, sanitation, and food-system infrastructure, low- and middle-income regions experience the highest per-person burdens (Iskandar et al., 2021; WHO, 2024). Many hospitalizations and deaths caused by tainted food occur due to the most common foodborne infections, which include *Salmonella* spp., *Escherichia coli*, and *Listeria monocytogenes* (Thomas et al., 2024). As recent methodological reviews have shown, case counts alone do not provide a complete picture of the public health impact. AMR among foodborne bacteria increases the probability of a complex and expensive illness, and some cases have serious sequelae, including reactive arthritis and haemolytic-uremic syndrome (Mody et al., 2021; Cribb et al., 2024).

Case studies that compare different areas further highlight the variations in FBD prevalence and its effects on public health. In order to trace the causes of infectious disease outbreaks and the development of antibiotic resistance, high-income nations like the United States and European Union heavily use molecular surveillance systems incorporating artificial intelligence and machine learning (Bilal et al., 2025). On the other hand, while a few metagenomic approaches exist in Malaysian research, culture-based methods are still the mainstay. This means new and quickly evolving diseases cannot be detected as easily (Quek et al., 2025). In parallel, prominent Japanese retailers such as Aeon have implemented

barcodes and Radio Frequency Identification (RFID) to inform customers of the origin and quality of their food, thereby enhancing the efficacy of food safety management response mechanisms (Zuo et al., 2022). These international benchmarks underscore the necessity of using molecular monitoring technologies and risk-based surveillance to enhance disease surveillance in Sarawak and throughout Malaysia.

2.2.1 Epidemiological Trends of Foodborne Diseases in Southeast Asia and Malaysia: Incidence, Drivers, and Public Health Implications

At the regional level, Southeast Asia has a significantly higher burden of FBDs than the global average, which is attributed to the concentrated population centres large informal food sectors, and climatic factors that facilitate the survival and proliferation of pathogens. Comparative analyses and national burden estimates suggest that Southeast Asia has one of the highest rates of infectious diseases, although the precise per-country estimates are contingent upon the completeness of reporting and the capacity of surveillance (Obnial et al., 2024). In addition, systematic reviews of the region have identified recurring drivers of outbreaks, including street-vended foods and mass-catering events, time-temperature abuse, and inconsistent food-handler training. These factors are epidemiologically significant in numerous Southeast Asian countries (Hassan et al., 2023). The results of these studies indicate that regional strategies must integrate behaviour-centered interventions for vendors and mass-catering operations with environmental (water, cold chain) enhancements.

The enduring national concern of FBDs is confirmed by Malaysia's published and administrative data. As per the Malaysia Annual Report Food Safety and Quality 2021, there was a consistent increase in the number of food poisoning cases between 2017 and 2019. Aspian et al. (2024) reported that the number of cases increased from 401 in 2017 to 496 in

2018 and finally peaked at 516 in 2019. However, the number of cases decreased to 288 in 2020 and 123 as of September 2021. A national study conducted from 2004 to 2014 estimated an average incidence of approximately 561 food-poisoning cases per 100,000 population and identified temperature and seasonal patterns as significant predictors of outbreak occurrence (Hassan et al., 2023). This finding takes on much more significance when taking Malaysia's tropical climate into account (Hassan et al., 2023). Academic evaluations that span longer time periods provide a more comprehensive picture. Many common bacteria found in store-bought and pre-prepared foods include *Salmonella*, *Staphylococcus aureus*, *Vibrio* spp., *Bacillus cereus*, and pathogenic *E. coli*. Peer-reviewed reviews of bacterial FBDs from Malaysia have shown an increasing prevalence of antibiotic resistance among these isolates (Cheesman et al., 2023). The results highlight the need for more frequent FBDs and AMR surveillance as part of Malaysia's regular food safety monitoring.

Research comparing Malaysia to global standards reveals striking differences. Peninsular Malaysia has a larger concentration of researchers and more frequent use of cutting-edge molecular techniques like genomic surveillance and shotgun metagenomics, making it easier to find genes that encode antibiotic resistance and profile foodborne pathogens in greater depth. Because of this, urban supply chains have been able to use risk-based inspection programs and receive targeted interventions (Quek et al., 2025). On the other hand, research in Sarawak is currently more restricted. The majority of recent studies (2023–2025) have concentrated on Ramadan bazaars and street markets in Kuching, demonstrating elevated aerobic plate counts and the frequent detection of *B. cereus*, *S. aureus*, and coliforms where some isolates have demonstrated resistance to common antibiotics (Azmi et al., 2023; Azmi et al., 2025). Although bazaar studies are beneficial for

identifying acute, event-based risks, their episodic and geographically limited nature means that the statewide prevalence, contamination pathways in institutional kitchens, and the broader resistome remain under-characterized. These gaps restrict direct comparison with Peninsular evidence and with international best practice, which employs continuous genomic surveillance in many high-income countries.

2.3 Microbiological Contamination in Food Premises

The continuation of hygiene challenges in Malaysian food premises is underscored by recent enforcement data. Notably, the Ministry of Health's Food Safety and Quality Division ordered 910 closures out of 63,784 inspected premises during the first half of 2024, a closure rate of approximately 1.4%. This underscores the persistent non-compliance issues that persist despite the existence of extant regulatory frameworks (Radhi, 2024). Ramadan bazaars and other seasonal markets are particularly scrutinized: in 2024, 1,639 hygiene and food-safety notices were issued nationwide, indicating targeted interventions in high-risk, high-density public food settings (Easter, 2024). At the same time, peer-reviewed microbiological investigations in Peninsular Malaysia have documented the increasing prevalence of AMR and the pervasive contamination of food systems. A study conducted in 2023 identified multidrug-resistant *Salmonella enterica* and *Staphylococcus aureus* in meat, dairy, and ready-to-eat products, suggesting that consumer-facing products are at risk of severe AMR threats (Elbehiry et al., 2025). Additionally, processing environments are implicated: a study conducted in Selangor revealed the presence of virulent, AMR *Salmonella serovars* in retail beef and poultry, emphasizing the risks associated with both the preparation and retail phases (Shafini et al., 2017). Agricultural sources are also significant; a study conducted by Osman et al. (2021) along Malaysia's East Coast (Kelantan,

Terengganu, and Pahang) reported a high prevalence of *Salmonella* (46.3%) in poultry cloacal swabs. This strain is frequently resistant to antibiotics such as sulfonamides (52%), tetracycline (39.5%), and others, underscoring the existence of AMR reservoirs at the farm level. This vertically integrated AMR challenge, which spans from farm to table, is collectively illustrated by the agricultural, processing, and retail findings.

In addition, research has begun to focus on environmental contamination, which includes food-contact surfaces. According to Zulfakar et al. (2018), a survey of university cafeteria facilities in Peninsular Malaysia revealed that utensils and cutting boards contained high levels of *E. coli* and *Salmonella*, with a limited correlation to inspection grades. This finding implies that surface contamination may persist despite clear sanitation measures. Additional innovation was introduced with the pilot of a rapid flow-cytometry detection method (CytoQuant®) that is capable of detecting microbial contamination on surfaces in under 30 seconds. This method is a significant advancement in the enforcement capability of busy or long-operating premises (Wahab et al., 2025). Integrating this technology into HACCP verification can enable food establishments to promptly identify contamination risks, thereby decreasing the probability of pathogen persistence and cross-contamination. Nevertheless, in order to guarantee scalability across various food service environments, these tools necessitate additional validation under operational conditions, cost-effectiveness assessments, and regulatory endorsement.

2.3.1 Comparative Analysis of Microbiological Contamination in Food Premises:

Sarawak, Peninsular Malaysia, and Southeast Asia

Recent enforcement activity in Sarawak illustrates active local supervision; however, there is still a scarcity of peer-reviewed evidence regarding environmental reservoirs of

contamination. For instance, municipality and divisional operations in Sibu and other districts have resulted in the seizure of noncompliant utensils, the closure of premises, and the issuance of numerous compound notices, suggesting that visible sanitary breaches are identified and addressed (Wong, 2025). Nevertheless, the majority of the microbiological research published in the region focuses on end-product testing, such as the foods sold at Ramadan bazaars, rather than systematic sampling of food-contact surfaces or premises (Azmi et al., 2023; Mohamad et al., 2024).

On the microbiological front, a study on foods sold at Ramadan bazaars in Kuching revealed *Bacillus cereus* in 21% (32/155) of the food samples, indicating a potential risk of toxin-mediated foodborne illness during high-density, festival-driven vending activities (Azmi et al., 2023). An additional investigation assessed the probability of microbial contamination in noodle samples (Mohamad et al., 2024). This study reported elevated aerobic plate counts, with 88% exceeding acceptable limits, as well as contamination with *E. coli* (21%), *S. aureus* (6%), and *B. cereus* (16%). However, the study did not detect *Salmonella* spp. These discoveries underscore the potential hazards of microbial contamination in bakery-type or noodle-based products, which are frequently encountered in bazaar environments (Mohamad et al., 2024). These product-level results demonstrate real exposure risk for consumers at high-throughput events but do not attribute contamination to specific environmental or handling vectors.

In contrast, the evidence basis from Peninsular Malaysia is more methodologically diverse and broader, encompassing farm, processing, retail, and some environmental sampling. Foodborne pathogens and emergent AMR have been repeatedly reported in studies conducted in Peninsular states, spanning multiple nodes of the food chain. For instance,

virulent AMR *Salmonella* isolates from retail and processing environments (Syed Abu Thahir et al., 2023) and multidrug-resistant non-typhoidal *Salmonella* in battered foods (Sandrasaigaran et al., 2023) offer more conclusive evidence in favour of farm-to-fork transmission hypotheses and targeted interventions. This expanded purview enables the identification of systemic AMR reservoirs and processing-level contamination, which have not yet been addressed in Sarawak's product-focused literature.

The relative emphasis placed on food-contact surfaces is a consequential methodological distinction. Food-contact surfaces have been investigated as critical cross-contamination points and proposed operational tools for routine monitoring in peninsular studies and pilot methods (such as rapid flow-cytometry assays). These approaches enable proactive remediation prior to consumer exposure. Systematic food-contact surface surveys are absent in recent peer-reviewed outputs in Sarawak (2020–2025), resulting in a surveillance vacuum. Contaminated food items can be identified, but the anatomic source of contamination, such as raw input, handler practices, or persistent surface biofilms, has not been investigated. Addressing this shortcoming would significantly enhance the attribution and control strategies.

Meanwhile, the recent field studies conducted in Indonesia and Thailand across Southeast Asia offer instructive empirical contrasts by explicitly integrating handler behaviour, environmental sampling, and microbiological testing. Microbiological contamination including *E. coli* in prepared dishes was associated with low food safety knowledge and training among handlers in a study of mass-catering operations in Yogyakarta, Indonesia. The dominant risk drivers were poor formal training and weak procedural compliance (Palupi et al., 2024). Similarly, research on street-food and vending

contexts in Thailand discovered coliform and *E. coli* contamination in a measurable fraction of food and water samples (Yongyod et al., 2023). The majority of stalls were documented to have inadequate water and equipment sanitation, which is evidence that links environmental and operational factors to measured contamination (Yongyod et al., 2023). As demonstrated by these studies, the combination of handler knowledge-attitude-practice data with microbiological assay results in actionable intervention points (training, water, and equipment sanitation).

A critical technical distinction between Sarawak, Peninsular Malaysia, and several Southeast counterparts is the extent of surveillance on food contact surfaces. Studies conducted outside of Sarawak have sampled cutting boards, utensils, and preparation counters, and they have shown that food contact surfaces frequently contain indicator organisms and pathogens that contribute to cross-contamination (Zulfakar et al., 2018; Yongyod et al., 2023; Zaki et al., 2024; Palupi et al., 2024; Wahab et al., 2025). Additionally, pilot work in Malaysia has been conducted to test rapid detection technologies for surface monitoring (Wahab et al., 2025). However, Sarawak's research from 2020 to 2025 consists of essentially no systematic, peer-reviewed food contact surface surveys. Consequently, the empirical chain from contaminated food item to surface or handler to corrective action is largely uncharacterized on a local scale. Public health authorities are compelled to rely on end-product detection and visible hygiene transgressions to guide enforcement in the absence of data on food contact surfaces, which restricts the adoption of targeted preventive measures.

The methodological discrepancies are significant for epidemiology and policy formulation. In regions where nations or studies amalgamate agricultural, processing,

environmental, and food handler data, such as in segments of Peninsular Malaysia, Indonesia, and Thailand, it becomes practicable to advocate for multi-faceted interventions, including AMR stewardship on farms, HACCP/process sanitation at processing facilities, cleaning protocols for food contact surfaces, and training for handlers in retail settings. The existing data in Sarawak substantiates consumer-level actions, such as premise closures and hygiene notifications, but lacks the robustness to warrant upstream or surface-targeted interventions with equivalent confidence. The monitoring in Sarawak is presently reactive and product-focused, whereas more comprehensive regional studies have facilitated proactive, source-specific suggestions.

2.4 Antimicrobial Resistance (AMR) in Foodborne Pathogens

The public health concern of AMR in contaminated pathogens has grown significantly in Malaysia, with Peninsular Malaysia exhibiting a more extensive research coverage than Sarawak. The profiles of AMR have been the subject of numerous studies in Peninsular Malaysia, spanning a variety of sectors such as poultry, aquaculture, and ready-to-eat (RTE) foods. In a study conducted in HACCP-certified poultry processing plants and RTE establishments in the Klang Valley, *Salmonella* isolates exhibited high resistance levels, including 100% resistance to ampicillin and over 80% resistance to chloramphenicol, as well as multidrug resistance in *Staphylococcus aureus* (Subramaniam et al., 2023). Besides that, between 2016 and 2018, genomic surveillance of *Salmonella enteritidis* revealed a nearly universal presence of aminoglycoside resistance genes, pervasive tetracycline resistance (45% overall), and ampicillin resistance in poultry and human isolates (Zakaria et al., 2021). The presence of multiple resistant pathogens in Malaysian markets was also confirmed by a meta-analysis of retail seafood (Odeyemi et al., 2023). Additionally, a study introduced

metagenomic approaches for detecting resistance genes in produce, indicating the growing sophistication of national AMR monitoring (Quek et al., 2025).

According to a study by Lihan et al. (2021), the aquaculture and aquatic environments of Sarawak are significant AMR reservoirs with gastrointestinal relevance, where plasmid-mediated antibiotic-resistant *Escherichia coli* were observed in rivers and aquaculture farms in northwest Borneo (including Sarawak). This study highlights the potential for mobile resistance genes to propagate in settings that interface with the food chain. Building on that environmental signal, Elexson et al. (2023) conducted a similar study that sampled three shrimp farms in the vicinity of Kuching. They discovered multidrug-resistant *Vibrio parahaemolyticus* in the shrimp, water, effluent, and sediments. Antimicrobial susceptibility testing confirmed multiple non-susceptibilities and multiple antibiotic resistance indices, which are indicative of high-risk antibiotic exposure in farm systems. Complementary surveillance conducted along the rivers of Bintulu revealed antibiotic-resistant heterotrophic bacteria with a significant degree of diversity. This finding supports the notion that local waterways can support resistant populations that may interact with fisheries and aquaculture (Leong et al., 2023).

The evidence basis is more limited on the consumer-facing side, but it is beginning to develop. A study conducted by Azmi et al. (2025) on beverages from Ramadan bazaars in Kuching identified *E. coli* and *Staphylococcus aureus*, as well as other *Enterobacteriaceae*, that were resistant to ampicillin/ceftazidime (for *Enterobacteriaceae*) and penicillin (for *S. aureus*). However, several isolates remained susceptible to gentamicin, chloramphenicol, tetracycline, ciprofloxacin, and trimethoprim/sulfamethoxazole (Azmi et al., 2025). Another foodborne organism of concern in informal retail contexts was identified in a related study

conducted in Kuching which evaluated *Bacillus cereus* contamination in bazaar foods and included susceptibility profiling of isolates (Azmi et al., 2023). In spite of the limited availability of detailed transmission mapping, these studies collectively illustrate the presence of AMR-associated hazards in regions where consumers purchase ready-to-eat items.

In Sarawak, a distinct research gap persists in spite of these advancements: there are no recent, peer-reviewed studies that systematically conduct antibiotic susceptibility testing on bacterial isolates recovered from (a) food-contact surfaces in food premises and (b) HACCP-certified food establishments in the state. There is a lack of standardised antibiotic susceptibility testing for swabs from high-touch surfaces, such as cutting boards, knives, prep tables, and serving utensils within regulated kitchens, and there is no evaluation of the effectiveness of HACCP implementation in Sarawak in suppressing resistant organisms on premises surfaces. Currently, Sarawak studies are focused on aquaculture and informal food markets. The significance of this gap is that food contact surfaces are widely recognised as vehicles for cross-contamination, and HACCP certification is designed to manage hazards during the preparation and service of food. In the absence of Sarawak-specific antibiotic susceptibility testing data, public health and food safety authorities are unable to confirm whether resistant strains are being managed at the point of food handling.

2.4.1 Drivers of AMR in Foodborne Pathogens

In Malaysia, AMR in foodborne pathogens is predominantly driven by a variety of interconnected factors, while patterns in Sarawak reflect similar yet more localised dynamics. The extensive use of antibiotics in food-producing animals and aquaculture, which is frequently for therapeutic, prophylactic, or growth-promoting purposes, is one of

the most significant drivers of the spread of AMR (Milijasevic et al., 2024). In the meantime, antibiotics such as aminoglycosides, tetracyclines, sulfonamides, and fluoroquinolones are frequently employed in aquaculture, occasionally without the necessary veterinary supervision (Au-Yeung et al., 2025). Such actions aid natural selection, which in turn promotes the evolution of bacteria that are resistant to antibiotics. These strains have the potential to contaminate food and water systems over time. The spread of antimicrobial resistance is accelerated by environmental contamination. Antibiotic contamination of rivers and other water sources is an issue. Untreated wastewater, agricultural discharge, and hospital effluents are among the sources where these residues can be detected (Chu et al., 2023). Numerous heterotrophic bacteria with antibiotic resistance can be found in the waters of Bintulu, Sarawak. Leong et al. (2023) found that ciprofloxacin and piperacillin failed to kill over 60% of the bacterial isolates tested. Particularly in rural and non-urban areas with possibly inadequate effluent treatment, the results demonstrate the importance of environmental pollution in developing and preventing antibiotic resistance. For Sarawak, aquaculture is an essential industry. Aquaculture farms in Borneo had highly resistant bacterial strains in their water, sediment, fish, and prawn samples. According to Kathleen et al. (2016), the high levels of antibiotic resistance were indicated by multidrug resistance indices exceeding 0.63. The potential for antibiotic-resistant bacteria to transmit their genes is a cause for alarm. Moreover, aquaculture farms and rivers in Sarawak were found to be home to plasmid-mediated antibiotic-resistant *Escherichia coli* (Lihan et al., 2021). The results emphasize the need for mobile genetic components to help bacteria in aquaculture and the surrounding environment pass resistance traits on to one another.

Antibiotic abuse is worsened by a lack of regulation and education. Although most Malaysians are aware of the risks posed by antibiotic resistance, about 70% of the populace

is unaware that these drugs find their way into cattle feed (Fatokun et al., 2024). Malaysia does not maintain an exhaustive database of authorized antibiotics for use in aquaculture and cattle, even though several states, like Sarawak, have small-scale and semi-commercial agricultural companies (Mariappan et al., 2021). Antibiotic use and emerging resistance trends are difficult to connect due to a lack of coordinated monitoring and surveillance. Finally, another factor contributing to the persistence of AMR is the limited implementation of a One Health approach, which considers the welfare of all living things. Despite having national AMR action plans, resistance profiling is not a priority in Malaysia's fisheries and aquaculture industries; instead, veterinary medication residues are the primary targets for monitoring. Food contact surfaces, food premises, and HACCP-certified food operations are mostly unknown in environmental studies particularly in Sarawak. These scenarios require extra care because they allow resistant bacteria to enter food that is already prepared to eat.

2.4.2 The Impact of AMR on Human and Animal Populations

AMR is a multifaceted issue that poses a threat to mankind and creatures. According to Ahmed et al. (2024), the development of drug-resistant bacteria is considerably influenced by the improper utilization of antibiotics in a variety of sectors, including agriculture, veterinary medicine, and healthcare. This has led to an increase in the prevalence of drug-resistant bacteria. Due to excessive antibiotic use, certain bacteria have acquired antibiotic resistance; these infections are referred to as "superbugs." Ahmed et al. (2024) conducted research that indicates that these bacteria have the potential to cause severe illnesses and substantially impede the efficacy of current treatments. The stagnant development of novel antimicrobial drugs is contributing to the escalation of the situation. The stagnation in the development of novel antimicrobial drugs is causing the situation to

worsen. Compared to effective therapy identification, the rates of resistance development are significantly higher.

Major clinical concerns have also arisen as a result of the growing intractable character of diseases that were previously treatable. Second and third line antibiotics have become more common as first-line antimicrobials have become less effective. These medications are more expensive, require extended treatment periods, and have more severe side effects (Soni et al., 2024; Ahmed et al., 2024). Economic productivity is also influenced by the length of recoveries, which results in additional time off work. In the absence of effective antibiotics, the limited ability to control infections could render routine procedures such as surgery, organ transplantations, chemotherapy, and neonatal care exponentially more hazardous (Ahmed et al., 2024). The most problematic multi-drug resistance threats that hospitals are currently facing are certain pathogens that are referred to as "ESKAPE" bacteria. These pathogens include resistant forms of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. (Soni et al., 2024). In a period without antibiotics, even little wounds or common illnesses spread by scratches might be deadly.

Resistance zoonotic pathogens can spread from animals to humans through AMR, endangering both human and agricultural health (Almansour et al., 2023). Reservoirs of resistance have emerged as a result of the over use of antibiotics in cattle for both medical purposes and growth promotion. Ahmed et al. (2024) found that this increases the likelihood of multidrug-resistant bacteria, such as *Salmonella* and *Campylobacter*, being transmitted through the food chain or by people who handle animals. Just like humans, animals can acquire AMR from unintended environmental exposures that cause the spread of other

pathogens. Fertilisers generated from manure spread resistant microorganisms into the wider environment, harming streams and the food that consumers eat. Furthermore, they interact with common environmental microbes and human commensal microflora, exchanging AMR genes (Ahmad et al., 2021). Consequently, in order to adequately handle the significant problem of AMR and its potential negative effects on animals, which increase the risks of human exposure, a One Health strategy that includes environmental, animal, and human health monitoring and interventions is still required (Mariappan et al., 2021; Ahmed et al., 2024).

2.5 Regulatory Framework and Certification Practices in Malaysian Food

Premises

The food-safety framework in Malaysia is founded on the Food Act 1983 and its subsidiary Food Regulations, which grant the Ministry of Health (MOH) the legal authority to conduct food-premise inspections, licensing, enforcement actions, and sample collection (Halim et al., 2024). In addition to the Act, the Food Hygiene Regulations 2009 classify food establishments into different types based on their involvement in the production and processing of food: P1 for manufacturing and processing, P2 for food service and catering, P3 for retail and small vendors, and P4 for food trucks that sell ready-to-eat food (Winsynergy, 2023). The MOH also advocates for formal food safety management certification programmes, including HACCP and Good Manufacturing Practices (GMP), within this legislative framework. This initiative is predicated on the national HACCP standard, Malaysian Standard (MS) 1480:2019, while Food Safety is the Responsibility of the Industry (MeSTI) and Clean, Safe, and Healthy (BeSS) provide tiered, industry-specific certification schemes to incentivise compliance across micro, small, and medium enterprises

(MSMEs). This combination of standards and legislation offers regulatory authorities the requisite legal tools to enforce compliance, while also encouraging larger and export-oriented firms to implement documented HACCP systems.

Compliance with mandated baseline certifications is a required condition for the legal operation of P2 establishments, which encompass kitchen hostels, cafeterias, catering outlets, canteens, and food courts. These requirements encompass mandatory food premise registration by the MOH through the Food Safety Information System of Malaysia (FoSIM). The preventive and documentation-oriented logic of HACCP is extended into routine regulatory oversight by FoSIM, which predominantly functions as a registration platform (MISHU editorial team, 2025). FoSIM allows regulators to conduct risk-based inspections and monitor sanitation performance across outlets by mandating that operators submit premise details, maintain records of food-handling activities, and document compliance status. FoSIM functions as a digital mechanism that is consistent with emphasis of HACCP on traceability, monitoring, and documentation, even for establishments that do not implement full HACCP certification.

Local authorities require that P2 premises obtain a genuine premise licence and signboard licence in addition to FoSIM registration to guarantee that zoning, structural safety, and sanitation standards are met. Regulation 30 of the Food Hygiene Regulations 2009 mandates that all food handlers must complete MOH-approved food handler training at the operational level. Certificates are now issued on a lifelong basis following the 2024 regulatory update. Typhoid vaccination is also mandatory for food handlers, and proof of vaccination must be retained for inspection purposes (MISHU editorial team, 2025). Periodic health inspections conducted by the MOH or municipal health officers are also required for

premises. These inspections encompass hygiene, waste management, pest control, and the cold-chain management of perishable foods (MISHU editorial team, 2025). Collectively, these certifications including MeSTI and BeSS establish the legal standard for food safety in consumer-facing establishments.

Research suggests that the Malaysian food sector is inconsistent in its adoption of advanced voluntary certification systems, such as HACCP, despite the existence of this robust framework. MSMEs, as well as informal food vendors, continue to rely heavily on fundamental Good Hygiene Practices (GHP) or ad hoc standard operating procedures. This uneven uptake is frequently attributed to barriers such as certification costs, limited technical capacity, and insufficient management commitment (Razak & Daud, 2020; Dima et al., 2024). As a result, HACCP certification is primarily obtained by larger organisations, hotels, and export-oriented processors that are subject to more stringent market or regulatory requirements.

The value of HACCP certification is demonstrated through empirical research, which also highlights its limitations. In comparison to uncertified outlets, microbiological assessments of HACCP-certified hotels and restaurants revealed that certification was associated with enhanced control of critical microbiological indicators. Nevertheless, the same studies demonstrated that certification alone does not ensure complete compliance, as continuous monitoring, verification, and staff retraining are necessary to maintain improvements (Lai et al., 2024).

Recent KAP studies have emphasised persistent disparities among food handlers at the operational level, even in establishments that satisfy baseline certification requirements. In practice, lapses are prevalent, although the majority of handlers exhibit moderate to

excellent theoretical knowledge regarding food safety (Aspian et al., 2024; Rohin et al., 2024). In lesser facilities, deficiencies are most apparent in areas such as temperature control, systematic record keeping, cross-contamination prevention, and correct handwashing techniques (Aspian et al., 2024; Rohin et al., 2024). These results indicate that mandatory certifications are insufficient to guarantee consistent safe practices in isolation, although they establish minimum compliance standards.

Scholars and practitioners are advocating for risk-based approaches that encompass more frequent refresher training, simplified checklists, and targeted inspections that concentrate on the highest-risk products and processes in order to reconcile this disparity. In this regard, the regulatory framework for P2 establishments is a dual system. On the one hand, a uniform baseline for public health protection is established by mandatory certifications, such as premise licencing, FoSIM registration, food handler training, and typhoid vaccination as well as MeSTI and BeSS certification. Conversely, HACCP and Halal certification are voluntary programmes that encourage greater conformance among businesses that are export-oriented, brand-conscious, or religiously compliant. Certification and standards have demonstrably improved food safety in formal, larger-scale venues, but they have produced more limited improvements in informal and small-scale sectors, which is explained by this duality.

2.5.1 Comparative Analysis of HACCP Implementation and Food Hygiene Research in Peninsular Malaysia and Sarawak: Evidence Gaps and Policy Implications

Empirical research in Peninsular Malaysia has evolved from policy descriptions to outcome-oriented studies that integrate microbiological testing with process audits. Subramaniam et al. (2023) report that multi-site audits of HACCP-certified dine-in and

hospital facilities in the Klang Valley generally demonstrate superior control of indicator organisms and improved temperature and hygiene records in certified settings. However, they also document residual implementation gaps, such as occasional cross-contamination or incomplete verification records. These peninsular studies therefore offer direct, measurable evidence that certification is correlated with enhanced process control. However, they also demonstrate that certification is insufficient in the absence of rigorous verification and staff-level compliance.

The Peninsular Malaysia evidence base is methodologically heterogeneous but relatively robust in three ways: (1) triangulation, which involves the integration of self-report, structured observation, and microbiological outcome data; (2) sectoral coverage, which involves audits of hospitals, food processors, and hospitality; and (3) linkage to risk management, which involves the explicit evaluation of the impact of HACCP/prerequisite program implementation on specific microbiological indicators, enabling regulators to prioritize verification actions (Subramaniam et al., 2023; Lai et al., 2024). Studies conducted in Peninsular Malaysia can combine process and outcome assessment to offer practical suggestions on the frequency of inspections, crucial control points, and staff training needs.

Contrarily, most of the Sarawak-related peer-reviewed literature is based on cross-sectional KAP surveys of food handlers, street vendors, and small food outlets. Only a handful of studies have used structured observation or microbiological endpoints (Mizanur et al., 2012; Jores et al., 2018; Elexson et al., 2023). Prior training, education, and outlet formality are significantly correlated with the heterogeneous knowledge and practice levels that are documented in numerous Sarawak investigations, including a compliance study that sampled the Kuching and Lawas sites. Reviewers of Malaysian consumer and vendor KAP

that encompass Sarawak observe that the state's published work prioritizes human-factor descriptions over performance audits of certified premises, and they explicitly emphasize the variability observed in urban and peri-urban settings (Mizanur et al., 2012; Jores et al., 2018). Consequently, descriptive KAP data in Sarawak are informative about behavioural determinants but do not provide the outcome metrics necessary to evaluate the effectiveness of HACCP at the facility level (Elexson et al., 2023).

The relative scarcity of peer-reviewed, outcome-oriented studies about HACCP-certified premises in Sarawak is a critical comparative lacuna. For instance, the Klang Valley HACCP audits and recent microbiological safety assessments in certified hotels/restaurants are examples of Peninsular studies that integrate environmental and product microbiology with process verification, thereby facilitating the direct comparison of certified and uncertified operations (Subramaniam et al., 2023). The scarcity of comparable Sarawak publications in the indexed/open literature that evaluate certified hotels, hospital kitchens, or processors using the same microbiological and process metrics restricts the capacity of state regulators and industry to ascertain whether certification results in comparable risk reductions in Sarawak's unique sectoral context, particularly seafood and aquaculture (Natrah et al., 2025). Although the literature gap is not inherently indicative of the absence of certified facilities, it is indicative of the absence of published performance evaluations.

This asymmetry in empirical evidence has practical implications. Targeted policy actions have been informed by the availability of outcome data in Peninsular Malaysia. For instance, the integration of process verification into remedial training, the emphasis on supplier verification for high-risk chains, and risk-based inspection scheduling. Policymakers in Sarawak are primarily dependent on KAP findings, which identify training

and infrastructure deficiencies among informal vendors. However, they are unable to calibrate HACCP verification efforts for certified premises or quantify the contribution of certification to the reduction of microbiological or AMR hazards in seafood value chains due to a lack of local microbiological evidence. Given Sarawak's significant aquaculture industry, this is especially significant because AMR and environmental contamination pathways necessitate farm-to-processor verification that incorporates both HACCP and One Health surveillance.

Therefore, the research and policy recommendations that result from this comparative analysis are dual and mutually enriching. First, conduct purposive, peer-reviewed audits of HACCP-certified hotels, hospital kitchens, and processors in Sarawak using the same sampling frames and microbiological indicators as those used in peninsular studies. These audits should include environmental swabs, product samples, and hand/food-contact swabs to ensure that the results are directly comparable. The second step is to continue and expand KAP research in Sarawak. However, it is important to reinforce the research by regularly incorporating structured observation and limited microbiological testing (indicator organisms) to validate self-reported practices and more effectively target behaviour-change interventions. These measures will collectively eliminate the evidence gap between Peninsular Malaysia and Sarawak and facilitate the evidence-based calibration of HACCP verification, training, and AMR mitigation strategies.

2.5.2 The Challenge towards Effective HACCP Implementation and Compliance

There Robust prerequisite programmes, including sanitation, apparatus maintenance and calibration, GMP, and supplier control, are essential for the successful implementation of HACCP. In the absence of these programs, HACCP plans are reduced to formal

documents rather than functional systems. A problem that undermines control strategies and monitoring plans is identified as frequent GMP non-conformances (facility layout, sanitation, and maintenance) as dominant causes of HACCP failure in small food plants by multiple systematic and field studies (Hasnan et al., 2022). This observation is further supported by global reviews that underscore prerequisites as the fundamental determinant of HACCP performance. Critical control points (CCPs) cannot be reliably monitored or verified in the absence of validated cleaning, calibrated instruments, and supplier assurance. For instance, despite the formal HACCP programs, field microbiological surveys of HACCP-certified foodservice and catering establishments in the Klang Valley revealed detectable coliforms and *Staphylococcus aureus* on ready-to-eat foods, contact surfaces, and hands (Subramaniam et al., 2023). This suggests that there are gaps in environmental monitoring, sanitation validation, and corrective-action implementation. Hasnan et al. (2022) have documented that small and medium enterprises (SMEs) frequently fail GMP checks in areas that are relevant to HACCP, such as maintenance and sanitation scheduling. This explains why certification alone has not yet resulted in uniformly safe microbiological outcomes across sectors.

The resource-and-capacity constraints that SMEs encounter are a regional and Malaysian phenomenon, and they serve as a crucial link between policy responses and observed non-conformances. The staged national framework in Malaysia establishes a distinct path for firms; however, the administrative burden of documentation, consultant fees, and cost continue to be significant obstacles that prevent numerous SMEs from advancing beyond basic certification (Dima, 2024). This is consistent with the results of research conducted in Indonesia and Vietnam, where a significant number of small processors prioritised business continuity over investments in formal food safety

management systems (FSMS). Additionally, the transaction costs of verification for smaller firms are exacerbated by subnational disparities in cold chain and lab access (Fajarwaty & Jukes, 2022; Van Durme et al., 2024). The operational hinge that differentiates nominal HACCP systems from functional ones is verification practice, which involves the routine acquisition and utilisation of environmental and microbiological data to initiate corrective action. Cross-country research indicates that HACCP is more paperwork-centric and reactive than preventive in regions with limited verification resources, such as regional laboratories, routine environmental sampling programs, and trained auditors (Awuchi, 2023). In Malaysia, the documented presence of indicator organisms in HACCP-certified settings is consistent with this pattern and suggests that the proximate policy lever for improving public-health outcomes is not merely increasing the number of certified operations but rather increasing verification capacity (Subramaniam et al., 2023).

The comparability of the national contexts in Southeast Asia underscores the distinct and shared challenges associated with the implementation of HACCP and related FSMS. Thailand and Vietnam, which are export-driven economies, have observed the successful implementation of HACCP by modern processors and significant exporters. However, micro and small operators continue to be hindered by their limited technical capacity and documentation burdens (Van Durme et al., 2024). The informal vending industry and uneven regional enforcement make applying HACCP in Indonesia and the Philippines more difficult, leading to a higher degree of variation there. Informal marketplaces, such as those involving street sellers and small-scale food businesses, pose a heightened threat to consumer health since they are difficult to regulate (Teves, 2015). Some areas of Indonesia cannot adequately oversee street sellers because of the decentralization of food safety duties (Fajarwaty & Jukes, 2022). Similarly, due to limited resources and a lack of understanding,

small-scale firms in the Philippines often do not have official HACCP certification (Teves, 2015). A lack of uniformity in food safety regulations worsens because enforcement is not centralized throughout governments. Singapore's dense laboratory capacity, more vigorous regulatory enforcement, and market incentives establish an "upper bound" for the region's HACCP effectiveness. Studies conducted in Singapore show that food-safety measures improve more consistently once FSMS is implemented (Aik et al., 2020), which supports the idea that coordinated and consistent enforcement and technical capacity are important in achieving food safety goals. The Philippines and Indonesia may learn something from the more prosperous countries of Vietnam, Singapore, and Thailand by implementing programs to strengthen local enforcement capacities, speed up certification procedures, and promote public-private partnerships.

When taken together, these results provide a consistent, evidence-based explanation for the variations between Malaysia and its neighbours: One factor determining the baseline is the level of maturity of prerequisites, such as GMP and sanitation systems. Another factor is the administrative burden of certification and the resource constraints of SMEs. Verification capacity, which includes labs, routine monitoring, and auditor competence, governs the transition from documentation to effective hazard control. Throughout the region, this three-factor model consistently corresponds with empirical studies in Malaysia (Subramaniam et al., 2023; Hasnan et al., 2022) and regional analyses (Aik et al., 2020; Van Durme et al., 2024), elucidating the reason why export-oriented, well-resourced processors outperform smaller domestic operators.

2.6 Gaps in Literature

Although Malaysia is increasingly focussing on food safety, there are still substantial research deficits, particularly in Sarawak. Microbiological surveillance of food contact surfaces, AMR monitoring in food service environments, and the assessment of preventive management systems, including HACCP, are the three primary domains in which these gaps are located. Compared to Sarawak, Peninsular Malaysia has a more robust evidence base, with the majority of Malaysian studies on foodborne contamination concentrating on ready-to-eat foods, agricultural produce, or uncooked meats. Nahar and Mahyudin (2018) have documented the contamination of utensils and cafeteria equipment with *E. coli* and *Salmonella* spp. in a study conducted in the Klang Valley. This study provides direct evidence of surface-mediated hazards. Conversely, research in Sarawak has primarily focused on informal food vendors and festive bazaars, with an emphasis on ready-to-eat food products such as noodles and beverages (Azmi et al., 2023; Mohamad et al., 2024; Azmi et al., 2025). Systematic studies that evaluate the microbiological quality of food contact surfaces, such as utensils, plates, glassware, and preparation tables, in P2 category food establishments, including hospital central kitchens, canteens, and catering outlets, are conspicuously absent due to this restricted scope. As these items are globally recognised vectors for cross-contamination, the absence of such surface-based surveillance creates a blind spot. Local authorities face significant challenges in the development of targeted sanitation protocols and the traceability of contamination pathways in institutional and commercial food service environments in the absence of empirical data.

The absence of antimicrobial resistance surveillance in food premises is a second gap. Despite Malaysia's significant progress in the characterisation of AMR in clinical

isolates (Mariappan et al., 2021), aquaculture systems (Natrah et al., 2025), and retail food products (Veloo et al., 2025), there is virtually no published data on the resistance profiles of bacteria recovered from food contact surfaces in P2 establishments in Sarawak, particularly. This omission is crucial because resistant strains that remain on utensils and service equipment can survive insufficient cleansing, infect ready-to-eat foods, and subsequently infiltrate the human population. The presence of multidrug-resistant *Salmonella* and *Staphylococcus aureus* in certified processing and food service environments has been demonstrated in Peninsular Malaysia (Subramaniam et al., 2023). Nevertheless, in Sarawak, AMR research has been primarily focused on aquaculture systems and festive food markets, with no systematic testing of surface isolates for multiple antibiotic resistance (MAR) indices (Lihan et al., 2021; Azmi et al., 2025). Therefore, the regional AMR burden is still unquantified as a result of the contribution of food service environments, which hinders the development of antimicrobial stewardship strategies and constrains the accuracy of risk assessment.

The evaluation of HACCP and related food safety management systems is the third of the gaps. Malaysia has implemented baseline programs such as MeSTI and BeSS, and it encourages HACCP certification. However, the efficacy of these systems in P2 category food establishments in Sarawak is not adequately documented. Outcome-based studies in Peninsular Malaysia have integrated microbiological testing with HACCP audits to demonstrate that certification enhances microbial control, but it does not ensure it (Subramaniam et al., 2023). On the other hand, the majority of available research in Sarawak has been limited to KAP surveys of food handlers, particularly among small-scale vendors and bazaar proprietors (Mizanur et al., 2012; Jores et al., 2018; Elexson et al., 2023). Seldom have peer-reviewed studies conducted a systematic audit of HACCP-certified P2

establishments, including cafeterias, hospital kitchens, or catering outlets, or compared compliance status with microbial outcomes. This discrepancy is alarming, as HACCP continues to be the preeminent preventive framework in national food safety policy. It is uncertain whether HACCP certification in Sarawak results in effective hazard reduction in practice, particularly in smaller or resource-constrained establishments, in the absence of reliable local evidence.

Finally, the current research in Sarawak tends to focus on microbial contamination, antimicrobial resistance, or food safety management practices in isolation, with minimal effort to integrate these dimensions. However, these risks are intrinsically linked: baseline hazard levels are established by microbial prevalence, clinical severity and treatment challenges associated with contamination are determined by resistance profiles, and the efficacy of preventive systems in mitigating these risks is revealed by HACCP implementation. Policymakers lack a comprehensive comprehension of the interactions between operational practices and microbial hazards and resistance dynamics in local food service environments due to the absence of integrated, multidimensional studies. In summary, the present evidence base in Sarawak is hampered by a lack of integrated, multidimensional assessments, inadequate surveillance of food contact surfaces, limited characterisation of AMR in food service environments, insufficient evaluation of HACCP performance in P2 establishments, and a lack of integrated, multidimensional assessments. It is imperative to address these deficiencies in order to support antimicrobial stewardship at the consumer-facing level of the food chain, guide risk-based inspections, and strengthen food safety governance.

2.6.1 Rationale for the research

In Sarawak, the convergence of three interrelated concerns, the microbial contamination of food contact surfaces, the increasing prevalence of AMR and inconsistent implementation of preventive food safety management systems has led to the rationale for this research. Collectively, these concerns pose a substantial public health challenge and underscore the necessity of conducting a comprehensive investigation into food establishments classified as P2 food establishments. Firstly, the microbiological quality of food contact surfaces in Sarawak is a subject of significant empirical data scarcity, despite their acknowledged function as vehicles for pathogen transmission. Despite the fact that infectious outbreaks in Malaysia have been consistently associated with deficiencies in hygiene practices, the majority of the existing research has concentrated on Peninsular Malaysia or has concentrated on ready-to-eat food products rather than environmental reservoirs such as utensils, plates, and preparation surfaces. The primary focus of peer-reviewed studies in Sarawak has been on informal vendors and festive bazaars, leaving institutional and commercial P2 establishments under-characterized. Regulators' capacity to develop context-specific hygiene interventions is impaired by this disparity, which also undermines the equitable implementation of national food safety standards throughout Malaysia. This investigation generates baseline data that are urgently needed for regional risk assessment by systematically quantifying bacterial prevalence on implements and surfaces in mass catering settings.

The second aspect of the research is that it directly addresses the growing global and national concern of antimicrobial resistance. A compounded threat is posed by resistant bacteria on food contact surfaces, as they enhance the probability of foodborne illness

outbreaks and simultaneously function as reservoirs that facilitate the persistence and dissemination of resistance characteristics within the community (Shafizi et al., 2024). Although Malaysia has implemented national AMR surveillance programs, these programs are primarily concentrated on clinical and agricultural contexts. The resistance profiles of bacteria isolated from food service environments, particularly in Sarawak, have been the subject of very few studies. By incorporating susceptibility testing and multiple antibiotic resistance (MAR) index analysis, this study addresses a critical void by offering evidence regarding the potential contribution of food service environments to the AMR burden. These results will help to reinforce antimicrobial stewardship strategies by directly connecting resistance surveillance to consumer-facing environments.

Third, the study assesses the implementation of HACCP in P2 establishments, a dimension that is rarely assessed in Sarawak. MeSTI and BeSS certifications establish baseline hygiene practices; however, they do not provide the structured hazard identification and monitoring that are essential to HACCP systems. Evidence from Peninsular Malaysia indicates that HACCP-certified establishments accomplish superior microbial control in comparison to uncertified outlets; however, there is no such evidence available for Sarawak. Certification in Sarawak may or may not lead to efficient risk management in practice; however, this is not known due to a lack of empirical research. This research provides a comprehensive overview of the operational and microbiological performance of P2 enterprises by combining microbial and resistance data with HACCP conformance assessments.

Combining the microbial prevalence, antimicrobial resistance profile, and HACCP evaluation enables a more comprehensive assessment than previous studies. By design,

surface contamination will not be investigated in a vacuum but in relation to its resilience characteristics and the control measures designed to lessen it. Using this method yields proper evidence that may be used by regulatory bodies to improve training programs for food handlers in Sarawak's food service sector, strengthen preventive frameworks, and prioritize enforcement. This study's results are helpful for more than just scholarly purposes; they can guide public health policy and pave the way for risk-based actions to safeguard consumers.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Area

The research had been carried out in P2 category food establishments that had been specifically selected for their locations in Kota Samarahan and Kuching, Sarawak. A total of thirty P2 category food establishments were selected. The criterion for selection in this study was the P2 category food establishment, as these premises were involved in mass catering, with samples collected from forks, spoons, tables, plates, and glasses from the food service outlets. The total amount of all samples was 150, 5 samples from food outlet with no triplicates. Triplicates were not taken as the samples had been analysed by using most probable number (MPN) technique. The following were used to represent these samples: spoons (n=30), forks (n=30), plates (n=30), glasses (n=30), and tables (n=30).

In order to mitigate potential bias in the selection of food premises, the outlets were selected based on clear and consistent inclusion criteria, specifically their classification as P2 category establishments that engage in mass catering. To prevent over-representation of a single district and assure geographical balance, the premises were further stratified by location, with approximately equal representation from Kuching and Kota Samarahan. The selection process was conducted without any preference for premises based on size, popularity, or customer flow, as this could potentially bias the results in favour of outlets with more resources. In addition, all surfaces that come into contact with food, such as spoons, forks, plates, glasses, and tables, were assigned equal sampling quotas to ensure consistency across all businesses. Regulatory frameworks like Clean, Safe, and Healthy

(BeSS) and Food Safety is the Responsibility of the Industry (MeSTI) were considered the foundational certification requirements for food safety measures in Malaysia. In order to maintain inclusivity across certification levels, HACCP-certified (Hazard Analysis and Critical Control Points-certified) establishments were added, even though they were rare in the research locations. By using consistent criteria and checking that representation was balanced across locations, the study reduced the likelihood of systematic bias in the data collection. Many different approaches to food safety management exist in Sarawak, and this strategy mirrors that.

3.2 Data Collection and Data Quality Assurance

The 150 samples obtained were tables and utensils that had been cleansed and prepared for use by customers in food service outlets. The utensils were swabbed in order to obtain microbial samples. The tip of each utensil and the surface of the tables were swabbed with sterilized cotton buds. Before swabbing, the cotton stick was sterilized and immersed in 10 mL of tryptic soy broth (TSB). Then, the cotton sticks were reinserted into the universal Bijou bottles containing 10 mL of TSB after swabbing. The samples were promptly labeled and sent to the laboratory for further analysis. A quantitative method was employed to evaluate the prevalence of pathogenic bacteria on various food contact surfaces from specific food establishments. The most probable number technique was employed with direct observation as a quantitative analysis method. The multi-antibiotic profile of positive samples is also being determined through a qualitative assessment. The antibiotic susceptibility testing was performed, necessitating the measurement of inhibition zones in positive samples for comparison with the antibiotic susceptibility criteria set out by the Clinical and Laboratory Standards Institute and the Food and Drug Administration (Wenzler

et al., 2023). Following this, a qualitative evaluation was performed, wherein this section utilized HACCP analysis through the hazard analysis worksheet to record probable risks (Aslani et al., 2024).

3.3 Most Probable Method (MPN) Method

The MPN technique was used to determine the bacterial concentration in every sample. 1 mL of each dilution was added to 9 mL of TSB after ten-fold serial dilutions were prepared to a dilution factor of 10^{-3} . The next step was to make three identical copies of each diluted sample by adding 1 mL of the sample to three small bottles, each holding 9 mL of TSB. Then, for 24 hours, triplicates of each sample were placed in an incubator set at 30°C. Both facultative anaerobic and aerobic bacteria could be cultivated in the nonselective TSB growth medium (Bonnet et al., 2020). Tubes were deemed positive if they showed signs of growth or turbidity changes, and negative if neither happened. Bacterial MPNs were determined by tallying the number of tested positive and negative tubes at each dilution and comparing them according to established statistical tables of MPN.

The MPN method did not target individual bacteria but rather growth units. According to Ilsan et al. (2024), for the MPN method to be effective, the sample had to be diluted to the point where no microorganisms could grow in the tube. One way to determine the quantity of microorganisms in the undiluted sample was to observe how they grew in dilution. Employing a laboratory-based MPN offers several advantages over a standard plate count: the results reveal the total microbial count, and specific microbial groups can be identified using appropriate selective and differential media. Furthermore, the data exhibited a higher likelihood of consistency between laboratories. Some limitations of the MPN technique include the extensive use of glassware or materials, the inability to observe the

morphology of microorganisms, insensitivity in quantifying the number of microorganisms, and the presence of inhibitory agents in the medium that impede microbial growth (Ilsan et al, 2024). The MPN denoted the estimated bacterial concentration within the tubes. Nonetheless, the test was just presumptive, and the identity of the bacterium remained undetermined. Consequently, tubes experiencing turbidity alterations or growth will be infected before plating. The bacteria must undergo 16s rRNA analysis to conclusively establish that the organism accountable for the alterations in the tubes was, in fact, the bacteria. This research necessitated 16s rRNA analysis, as the supplier requested these results prior to dispatching bacterial samples for sequencing, which would confirm the strains and identities of the bacteria. Disc diffusion analysis will determine the multi-antibiotic profile of the bacteria acquired by plating after their identities have been verified.

3.3.1 Enrichment and MPN Method

The MPN method was used to determine the microbial concentration in each sample (Nahar & Mahyudin, 2018). The dilution factor 10^{-3} was attained by making consecutive dilutions ten times stronger. Then, 9 mL of TSB was mixed with 1 mL of each dilution. After that, three identical bijou vials were filled with 9 mL of TSB each, and 1 mL of each diluted sample was transferred into each. The next step was to incubate three samples at 30°C for one day. According to Bonnet et al. (2020), facultative anaerobic and aerobic bacteria could be grown in the nonselective TSB growth medium. Generally, tubes were considered negative if they showed no signs of growth or turbidity changes, and positive if they did. Bacterial MPN was determined by recording the number of tubes that tested positive or negative at each dilution and referring to reference materials from traditional statistical MPN tables. The turbidity of the MPN tubes was evaluated before genomic DNA was extracted

following incubation (Lesen et al., 2024). This experiment also used sterile TSB broth as the negative control.

3.3.2 Most Probable Number-Polymerase Chain Reaction (MPN-PCR) and Genomic DNA Extraction

The boiled-cell method was employed to extract bacterial DNA from the turbid MPN tubes with minimal modifications (Lesen et al., 2024). 500 µL of each culture was transferred to a 1.5 mL microcentrifuge tube, and the tube was subsequently centrifuged at 10,000 rpm for 5 minutes. After removing the supernatant, 100 µL of sterile distilled water was used to resuspend the particle. Subsequently, the suspension was boiled for 10 minutes and chilled to -21°C for 5 minutes. The DNA extract was subsequently stored at -21°C for analysis after being centrifuged at 10,000 rpm for 10 minutes. The final supernatant that contained DNA would serve as the MPN-PCR template.

3.3.3 16s rRNA Sequencing

A pair of universal primers specifically engineered to target pathogenic bacteria was employed for 16S rRNA gene sequencing. A 1,400-bp 16S rRNA gene was amplified from all 69 bacterial DNA using the primers 27F (F 5'AGAGTTTGATCMTGGCTGAG-3') and 1492R (R 5' TACGGYTACCTTGTTACGACT T-3') (Chukwu et al., 2019). The following components were present in each 15 µl of the polymerase chain reaction (PCR) mixture: 7.5 µl of exTEN 2X PCR Master Mix (1st BASE), 0.6 µl of each primer with a concentration of 10 µM, 2.0 µL of DNA template, and 1.9 µL of sterile purified water are all that is necessary. The PCR was performed using the T100™ Thermal Cycler (Bio-Rad, USA) under the following conditions according to Chukwu et al. (2019): an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for

30 seconds, and extension at 72°C for 2 minutes. The final extension was performed at 72°C for 5 minutes and 4°C for an infinite hold. Electrophoresis was performed on a 1.5% (w/v) agarose gel with a 1 kb molecular size marker (Thermo Fisher Scientific, USA) with a 2 µl aliquot of each amplified product. The electrophoresis was performed at 90 V in Tris/borate/EDTA buffer for 1.5 hours. The bands were observed under the predicted 1.4 kb size after the gel was stained with ethidium bromide (EtBR) for 1 hour, rinsed, and photographed under ultraviolet light.

3.4 Disc Diffusion Test for Antibiotic Susceptibility Testing (AST)

The disc diffusion test was a qualitative test that permitted the growth of a bacterial lawn in the presence of antibiotic discs, followed by a 24-hour incubation period. The grey region surrounding the discs indicated the zone of inhibition of antibiotics. The objective of this test was to illustrate the multi-antibiotic profile of the bacterium. In the near future, this allowed physicians to employ the data efficiently to ascertain the most optimal therapy options. A zone of inhibition is an environment where antibiotic-resistant microorganisms thrive instead of those that are sensitive to antibiotics. The zone of inhibition of a bacterium must be quantified and compared to the antibiotic susceptibility table established by the Clinical and Laboratory Standards Institute (CLSI) and Food and Drug Administration (FDA) in order to determine whether it is susceptible, intermediate, or resistant to an antibiotic (Wenzler et al., 2023). Antibiotic susceptibility of foodborne bacteria was tested against six commonly prescribed and available antibiotics (ciprofloxacin, amikacin, kanamycin, imipenem, meropenem and ceftazidime).

Mueller Hilton Agar (MHA) was employed in AST due to its non-selective and non-differential nature. Consequently, the majority of the organisms would grow on this agar,

and there would be no bias when the zone of inhibition was measured. Additionally, MHA contained starch. The bacterial growth and antibiotic diffusion were not interfered with by starch, which acted as a toxin absorber (Team, 2024). Additionally, it served as an energy source for the microbes. Finally, MHA was a loose agar, which resulted in improved antibiotic diffusion (Team, 2024). The procedures in this test must be completed within 15 minutes to prevent cross-contamination. A control plate was required for the diffusion disc analysis. This was done to guarantee that the growth condition was appropriate, the growth time was adequate, and the zone of inhibition was accurate. The control plate was manufactured in the same manner as the testing plate. Nevertheless, the investigation employed a control organism, such as *E. coli* was used in the research. In order to verify that the bacteria had grown sufficiently, the control plate was examined after 24 hours. The zone of inhibition would be measured and compared to the sensitivity table. The testing plates were examined once the zone of inhibition of a control plate was determined to be accurate. If the quality controls failed, the testing plates would be deemed unacceptable and would not be reported.

3.5 Hazard Analysis and Critical Control Point (HACCP) Analysis

The HACCP analysis was conducted on P2 category food establishments in general. Inspection of food preparation processes, such as holding, heating, and chilling, for potential contamination by chemical, biological, and physical hazards was a component of the HACCP analysis (Abd Lataf et al., 2020). The potential hazards would be recorded using the hazard analysis worksheet during the assessment (Abd Lataf et al., 2020; Aslani et al., 2024). This assessment was qualitative in nature, as it merely recommended additional research into the potential source of contamination during the food production process. The HACCP

system standard (MS1480:2007) for food safety was followed in the development of the decision tree for identifying sensitive raw materials and the decision tree for determining critical control point (Abd Lataf et al., 2020). The scope of HACCP for this study encompassed the procurement of basic materials and the subsequent distribution of ready-to-eat (RTE) food to consumers (Abd Lataf et al., 2020). Figure 3.1 illustrates five categories of severity resulting from hazards: (i) fatality occurrence, (ii) serious illness, (iii) product recall, (iv) customer complaint and (v) not significant. The likelihood of hazards was also classified into five categories: (i) common repeating, (ii) known to occur, (iii) could occur, (iv) not expected to occur and (v) practically impossible. The implementation of control measures was required pursuant to a score of 1-10, which indicated a critical food safety concern, where the values of non-significant concerns would fall within the range of 11 to 25 (Abd Lataf et al., 2020).

This study implemented a structured and stratified sampling strategy to reduce selection bias in the selection of food premises. The inclusion of only P2 category establishments (medium-risk food service premises) ensured consistent results across all outlets (Winsynergy, 2023). P2 is classified as medium-risk due to the fact that food is prepared and managed on site, which is a higher risk than P1 premises. However, the scale and storage/holding conditions are less extensive than those of P3 premises. A comprehensive list of eligible premises was compiled by utilising the Food Safety Information System of Malaysia (FoSIM) to access registration records maintained by the Ministry of Health. Inclusion was limited to outlets that had verified compliance with HACCP certification, as well as baseline certification programs like MeSTI and BeSS. The certifications (BeSS and MeSTI) in Malaysia serve as "HACCP-lite" systems, establishing minimum hygiene and safety standards. This reduces variability in baseline food safety

performance and minimises bias introduced by uncertified outlets. Sampling across two administrative districts (Kuching and Kota Samarahan) also mitigated geographic bias, thereby capturing variability in both urban and peri-urban contexts.

	Likelihood				
Severity	A	B	C	D	E
1	1	2	4	7	11
2	3	5	8	12	16
3	6	9	13	17	20
4	10	14	18	21	23
5	15	19	22	24	25
Hazard severity:			Hazard likelihood:		
1. Fatality occurrence 2. Serious illness 3. Product recall 4. Customer complaint 5. Not significant			A. Common repeating B. Known to occur or 'it has happened' C. Could occur or "I've heard of it happening" D. Not expected to occur E. Practically impossible		

Figure 3.1: Food Safety Hazard Matrix according to MS1480:2007 (Abd Lataf et al., 2020)

3.6 Ethical Consideration

Ethical approval for this study was obtained from the Faculty of Resource Science and Technology of the University Malaysia Sarawak. The food establishments were selected according to their inclusion criteria (P2 category food establishments, locations etc.). Following discussions with the supervisor and reaching a final decision, the food establishments will be contacted to assess their accessibility and availability prior to data collection. Furthermore, the objectives and the procedures conducted in the study would be explained via emails, phone conversations, or in-person meetings if necessary. This study did not include any personal identifiable information of the respondents or the identities of the food establishments. Participation of respondents was voluntary, and their comments were handled with a high degree of confidentiality and anonymity.

3.7 Data Analysis

The assessment of microbial loads on food contact surfaces was performed utilizing Microsoft Excel (Microsoft Corp, Redmond, WA). The MPN values for detecting and enumerating foodborne pathogens were derived from the three-tube MPN table. The outcomes of the antibiotic susceptibility testing were also compiled into a table. Plasmids from positive colonies with a multiple antibiotic resistance (MAR) Index exceeding 0.2 were extracted for PCR analysis. The PCR products then underwent Sanger sequencing conducted by Apical Scientific Sdn. Bhd. (Serdang, Malaysia). The acquired sequences were analysed with CLC Sequence Viewer 8.0 for alignment purposes. The aligned sequences were exported as FASTA files, which were then analysed through BLASTp against a database to identify the bacterial species involved.

3.7.1 Statistical Analysis

In order to identify significant variations between food establishments and to compare the microbial loads on various food contact surfaces, statistical analysis was performed. Analysis of Variance (ANOVA) was used, and $p < 0.05$ was considered significant. IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA) was used for all analyses.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Concentration of Bacteria on Different Food Contact Surfaces

In total, 150 samples were collected from five different food contact surfaces (spoons, forks, tables, glasses and plates) from thirty food establishments. Table 4.1 presents the total data of microbial loads of bacteria collected from selected food premises. Bacteria were detected in 69 out of 150 samples (46%) using the most probable number-polymerase chain reaction (MPN-PCR) method. The estimation of microbial load from the samples collected from P2 category food premises in Kota Samarahan and Kuching, Sarawak ranged from 3 MPN/mL to more than 1100 MPN/mL (refer to Table 4.1). Bacteria were detected on nearly all food outlets, with the exception of food outlets 2 and 14.

Table 4.2 summarizes the rate of conformance and non-conformance and minimum and maximum MPN values across sample types. It was shown that the spoon samples (contamination rate=43.33%; non-contamination rate=56.67%) from food outlets 21, 22, 24, 25, 26, and 27 had the highest bacterial concentration, which exceeded 1,100 MPN/mL. At food outlet 17, the bacterial concentration was the lowest, measuring 3.6 MPN/mL. For fork samples, the bacterial concentration was highest at food outlets 7, 8, 12, 20, 22, 23, 24, 26, and 27, surpassing 1,100 MPN/mL. The fork samples from food outlet 21 contained the lowest concentration of bacteria, measured 3.6 MPN/mL. The contamination rate for fork samples was 40%, while the non-contamination rate was 60%.

Table 4.1: Concentration of bacteria on food contact surfaces in selected food premises

Sample	Premise (MPN/mL)															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Spoon (s)	Min ^a	-	-	-	-	-	-	18	-	-	-	-	-	-	-	90
	Med ^b	-	-	-	-	-	-	93	-	-	-	-	-	-	-	460
	Max ^c	-	-	-	-	-	-	420	-	-	-	-	-	-	-	2000
Fork (f)	Min ^a	-	-	-	-	-	-	420	420	-	-	-	420	-	-	-
	Med ^b	-	-	-	-	-	-	>1100	>1100	-	-	-	>1100	-	-	-
	Max ^c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Plate (p)	Min ^a	0.17	-	-	0.15	420	-	90	-	-	-	-	-	0.15	-	-
	Med ^b	3.6	-	-	3	>1100	-	460	-	-	-	-	-	3	-	-
	Max ^c	18	-	-	11	-	-	2000	-	-	-	-	-	9.6	-	-
Table (t)	Min ^a	42	-	-	-	18	-	42	420	420	1.4	1.4	-	18	-	-
	Med ^b	240	-	-	-	93	-	240	>1100	>1100	9.2	9.2	-	93	-	-
	Max ^c	1000	-	-	-	420	-	1000	-	-	38	38	-	420	-	-
Glass (g)	Min ^a	-	-	1.4	-	-	17	420	420	-	-	-	-	-	-	-
	Med ^b	-	-	9.2	-	-	75	>1100	>1100	-	-	-	-	-	-	-
	Max ^c	-	-	38	-	-	200	-	-	-	-	-	-	-	-	-
Sample	Premise (MPN/mL)															
		16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Spoon (s)	Min ^a	9	0.17	17	-	180	420	420	-	420	420	420	420	-	-	4.6
	Med ^b	43	3.6	75	-	1100	>1100	>1100	-	>1100	>1100	>1100	>1100	-	-	23

Table 4.1 continued

	Max ^c	180	18	200	-	4100	-	-	-	-	-	-	-	-	-	94
Fork (f)	Min ^a	-	-	4.6	-	420	0.17	420	420	420	-	420	420	-	-	4.6
	Med ^b	-	-	23	-	>1100	3.6	>1100	>1100	>1100	-	>1100	>1100	-	-	23
	Max ^c	-	-	94	-	-	18	-	-	-	-	-	-	-	-	94
Plate (p)	Min ^a	-	-	42	-	420	-	420	420	420	420	420	420	-	-	0.17
	Med ^b	-	-	240	-	>1100	-	>1100	>1100	>1100	>1100	>1100	>1100	-	-	3.6
	Max ^c	-	-	1000	-	-	-	-	-	-	-	-	-	-	-	18
Table (t)	Min ^a	-	-	-	0.17	420	-	420	-	420	420	420	420	9	1.4	-
	Med ^b	-	-	-	3.6	>1100	-	>1100	-	>1100	>1100	>1100	>1100	43	9.2	-
	Max ^c	-	-	-	18	-	-	-	-	-	-	-	-	180	38	-
Glass (g)	Min ^a	1.4	1.3	-	-	9	-	420	420	420	420	420	420	-	-	-
	Med ^b	9.2	7.4	-	-	43	-	>1100	>1100	>1100	>1100	>1100	>1100	-	-	-
	Max ^c	38	20	-	-	180	-	-	-	-	-	-	-	-	-	-

^a Minimum MPN mL⁻¹ value; ^b Median MPN mL⁻¹ value; ^c Maximum MPN mL⁻¹ value, - = No minimum or maximum value

*Premises 1-14 were located in Kuching, premises 15-30 were located in Kota Samarahan

Table 4.2: Rate of Conformance and Non-Conformance and Minimum and Maximum MPN Values Across Sample Types

Sample items	n	Rate of Non-Conformance	Rate of Conformance	Minimum MPN (MPN/mL)	Maximum MPN (MPN/mL)
Spoons	30	13 (43.33%)	17 (56.67%)	3.6	>1100
Forks	30	12 (40%)	18 (60%)	3.6	>1100
Plates	30	14 (46.67%)	16 (53.33%)	3.0	>1100
Tables	30	17 (56.67%)	13 (43.33%)	3.6	>1100
Glasses	30	13 (43.33%)	17 (56.67%)	7.4	>1100

Additionally, it was recorded that the plate samples from food outlets 5, 20, 22, 23, 24, 25, 26, and 27 contained the highest concentration of bacteria, more than 1,100 MPN/mL. In contrast, the plate samples from food outlets 4 and 13 had the lowest concentration of bacteria, which was 3.0 MPN/mL. The rate of contamination for plate samples was 46.67%, while the rate of non-contamination was 53.33%. Subsequently, the concentration of bacteria in the table samples was determined to be the highest at food outlets 8, 9, 20, 22, 24, 25, 26, and 27, with a value exceeding 1,100 MPN/mL. In contrast, the table samples from food outlet 19 had the lowest concentration of bacteria, measuring at 3.6 MPN/mL. 56.67% of the table samples were contaminated, while 43.33% were uncontaminated. Lastly, the glass samples from food outlets 7, 8, 22, 23, 24, 25, 26, and 27 exhibited the highest bacterial concentration, which exceeded 1,100 MPN/mL. At food outlet 17, the bacterial concentration in glass samples was the lowest, measured 7.4 MPN/mL. The non-contamination rate was 56.67%, while the contamination rate for glass samples was 43.33%.

Food outlets with the highest contamination were food outlets 7, 20, 22, 24, 26 and 27 with 100% contamination for all food contact surfaces' samples, meanwhile food outlets 2 and 14 had the lowest contamination rate at 0% (refer to Figure 4.1). For the remaining

food outlets, the contamination rate of food contact surfaces' samples were as followed: food outlets 3, 4, 6, 9, 10, 11, 12, 15, 19, 28 and 29 had 10% contamination rate; food outlets 1, 5, 13, 16, 17 and 21 had 20% contamination rate; food outlets 18, 23 and 30 had 30% contamination rate; food outlet 8 had a contamination rate of 60% and food outlet 25 had a contamination rate of 80%.

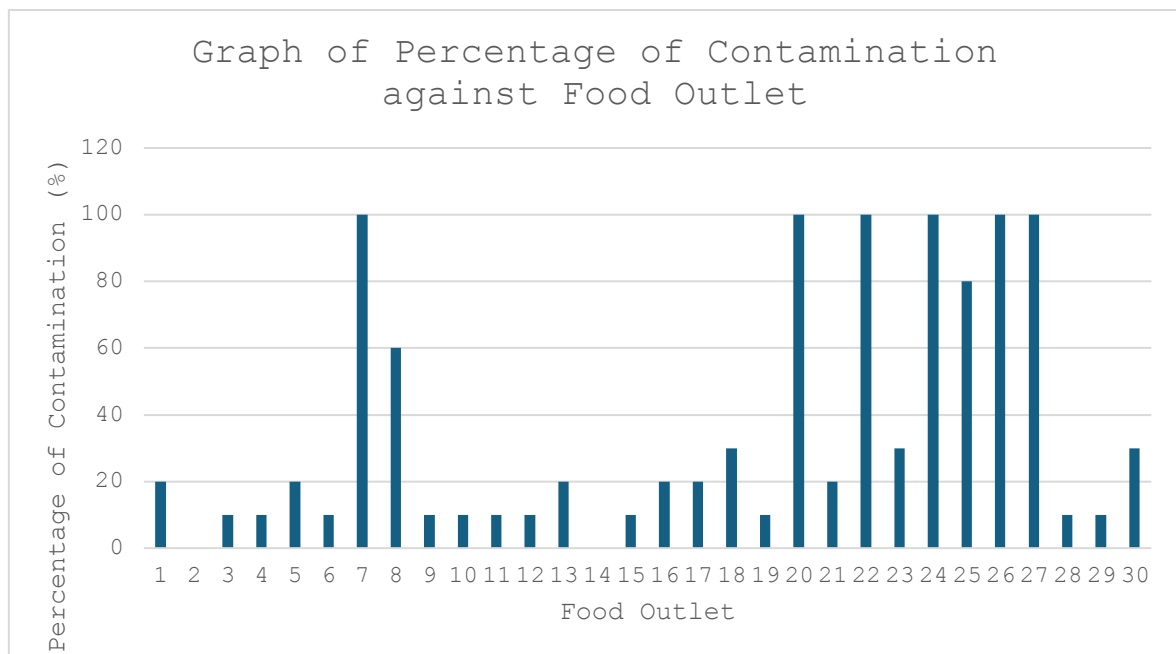


Figure 4.1: Graph of Percentage of Contamination against Food Outlet

4.1.2 Statistical Analysis for MPN Results

Table 4.3 displays the analysis of variance (ANOVA) test results for the MPN values in 30 P2 category food establishments. As shown in the table, the F-statistic was 10.57, which confirmed that the variability between the food outlets was significantly greater than the variability within the outlets. The p-value was also extremely small (2.31×10^{-21}) which was much less than the commonly used significance level of 0.05. Since the p-value was less than 0.05, the null hypothesis was rejected which meant that there was a significant difference in bacterial concentrations across the 30 food outlets.

Table 4.3: Anova Test for MPN values in 30 Selected Food Establishments

Source	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	F-Statistic	p-value
Between Groups	437245.4	29	15084.8	10.57	2.31×10^{-21}
Within Groups	60479.2	120	504.0		
Total	497724.6	149			

Table 4.4 displays the ANOVA test results for the MPN values in 14 food premises in Kuching (food outlets 1-14). As shown in the table, the F-statistic was 2.68, which confirmed that the variability between the food outlets was significantly greater than the variability within the outlets. Since the p-value (0.0054) was less than the significance level of 0.05, thus the null hypothesis was rejected. This demonstrated a statistically significant difference in the means of the MPN values among the 14 food outlets, suggesting variable bacterial concentrations in these establishments.

Table 4.4: Anova Test for MPN values for Premises (Food Outlets 1-14) in Kuching

Source	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	F-Statistic	p-value
Between Groups	676865.72	13	52067.37	2.68	0.0054
Within Groups	671456.12	56	11952.58		
Total	1343321.84	69			

Table 4.5 shows the ANOVA test results for the MPN values in 16 food premises in Kota Samarahan (food outlets 15-30). The F-statistic, as displayed in the table, was 17.24, confirming that there was substantially more variability between the food outlets than there was within them. It was decided to reject the null hypothesis since the p-value (5.49×10^{-17})

was below the significance level of 0.05. This showed that the means of the MPN values varied statistically significantly among the 16 food outlets, indicating that the bacterial concentrations in these food establishments varied.

The ANOVA test result in Kota Samarahan (refer to Table 4.5) demonstrated a significantly higher level of statistical significance than the ANOVA test result in Kuching (refer to Table 4.4). The difference between the food outlets in Kota Samarahan and Kuching was much greater, as evidenced by the F-statistic in Kota Samarahan (17.24) being significantly higher than in Kuching (2.68). Furthermore, p-value of result in Kota Samarahan (5.49×10^{-17}) was incredibly low, suggesting strong evidence against the null hypothesis, which proposed that the bacterial concentrations in the various food outlets varied significantly.

On the other hand, although Kuching also demonstrated statistical significance (p-value of 0.0054), the evidence was not as strong as that of Kota Samarahan, indicating that although there were variations in bacterial concentrations, they were not as noticeable. The ANOVA results concluded that, although both ANOVA test results in Kota Samarahan and Kuching showed significant bacterial concentration in their respective food establishments, but in comparison to Kuching, Kota Samarahan had a substantially greater and more noticeable variance in bacterial concentrations across outlets.

Table 4.5: Anova Test for MPN values for Premises (Food Outlets 15-30) in Kota Samarahan

Source of Variation	Sum of Squares	Degrees of Freedom (df)	Mean Square	F-statistic	p-value
Between Groups	7346625.02	29	253678.38	17.24	5.49×10^{-17}
Within Groups	4934510.91	120	41125.91		
Total	12241135.93	149			

4.1.3 Polymerase Chain Reaction (PCR) Result using 16s rRNA Sequencing

The expected size for the PCR products of foodborne bacteria in general is 1.4 kb (Chukwu et al., 2019). The agarose gel electrophoresis of the PCR products showed the presence of bands corresponding to the anticipated size (Figure 4.2). For targeted amplifications with the universal primer set, 27F and 1492R, an annealing temperature of 56°C proved to be optimal during optimization. Conversely, no band was observed during the agarose gel electrophoresis for PCR products that were annealed at temperatures below or above 56°C.



Figure 4.2: Gel electrophoresis of 16s rRNA sequencing of foodborne bacteria (1400 bp). Lane M: 1000 bp (1 kb) DNA ladder; Lane 1: negative control; Lane 2: positive control (*E. coli*); Lane 3-18: representative positive samples.

4.1.4 Species Identified among Selected Isolates

A total of 150 samples from food contact surfaces were analysed for the presence of foodborne pathogens. The study revealed 69/150 (46%) of the food samples had bacterial contamination (refer to Table 4.1). Every single isolate had a single genus that was associated

with it, which resulted in the highest percentage of identity score as shown in Table 4.5. The next nearest species had a percentage of identity score that ranged from 0.15 to 0.56.

From 150 contaminated samples, only 24 isolates with multiple antibiotic resistance (MAR) index more than 0.2 were identified (refer to Table 4.6). At the genus level, the most often recovered genus was *Enterobacter* (33.33%, n = 8). This was followed by *Klebsiella*, *Staphylococcus*, and *Exiguobacterium*, each of which was recovered at 12.5% (n = 3). *Enterococcus* and *Aeromonas* were recovered at 8.33% (n = 2), and *Bacillus*, *Alcaligenes*, and *Acinetobacter* were recovered at 4.17% (n = 1). As shown in Table 4.7, the highest numbers of bacterial isolates (n=24) with multiple antibiotic resistance (MAR) Index more than 0.2 were isolated from fork samples (n=7, 29.17%), followed by spoon and table samples respectively (n=5, 20.83%), glass samples (n=4, 16.67%) and plate samples (n=3, 12.5%).

Table 4.6: Breakdown of collected bacteria species and genera after Sanger Sequencing with MAR Index more than 0.2 and their percentage identity

Sample ID	Genus	Species	Percentage Identity (%)
6G	<i>Enterococcus</i>	<i>faecalis</i>	99.50
7T	<i>Bacillus</i>	<i>pumilus</i>	99.39
		<i>zhangzhouensis</i>	99.39
		<i>safensis</i>	99.39
12F	<i>Enterobacter</i>	<i>mori</i>	99.41
		<i>sichuanensis</i>	99.41
		<i>quasiroegenkampii</i>	99.41
18S	<i>Klebsiella</i>	<i>pneumoniae</i>	99.29
19T	<i>Staphylococcus</i>	<i>warneri</i>	100
		<i>pasteuri</i>	99.55
		<i>lugdunensis</i>	99.70
20S	<i>Enterobacter</i>	<i>cloacae</i>	99.62
		<i>asburiae</i>	99.62

Table 4.6 continued

		<i>mori</i>	99.43
20F	<i>Enterobacter</i>	<i>hormaechei</i>	95.10
		<i>cloacae</i>	95.10
		<i>quasihormaechei</i>	95.10
20G	<i>Staphylococcus</i>	<i>microti</i>	80.37
		<i>rostri</i>	80.37
		<i>muscae</i>	80.37
21F	<i>Staphylococcus</i>	<i>saprophyticus</i>	100
		<i>edaphicus</i>	100
22S	<i>Enterococcus</i>	<i>faecium</i>	100
		<i>thailandicus</i>	100
22F	<i>Exiguobacterium</i>	<i>indicum</i>	99.84
		<i>acetylicum</i>	99.68
22P	<i>Exiguobacterium</i>	<i>indicum</i>	96.40
		<i>acetylicum</i>	96.40
22G	<i>Exiguobacterium</i>	<i>indicum</i>	99.71
		<i>acetylicum</i>	99.71
24F	<i>Aeromonas</i>	<i>veronii</i>	100
		<i>bivalvium</i>	100
25T	<i>Klebsiella</i>	<i>africana</i>	100
		<i>pneumoniae</i>	100
		<i>quasipneumoniae</i>	99.68
25S	<i>Enterobacter</i>	<i>cloacae</i>	99.86
26F	<i>Alcaligenes</i>	<i>faecalis</i>	100
		<i>ammonioxydans</i>	100
		<i>pakistanensis</i>	99.44
26P	<i>Enterobacter</i>	<i>asburiae</i>	100
		<i>cloacae</i>	100
		<i>sichuanensis</i>	100
26G	<i>Enterobacter</i>	<i>asburiae</i>	99.50
		<i>cloacae</i>	99.50
		<i>hormaechei</i>	99.17
27F	<i>Enterobacter</i>	<i>sichuanensis</i>	99.55

Table 4.6 continued

		<i>quasiroggenkampii</i>	99.55
		<i>hormaechei</i>	99.40
27T	<i>Aeromonas</i>	<i>veronii</i>	99.53
		<i>rivipollensis</i>	99.37
27P	<i>Enterobacter</i>	<i>bugandensis</i>	100
		<i>hormaechei</i>	99.81
		<i>cloacae</i>	99.81
28T	<i>Klebsiella</i>	<i>quasipneumoniae</i>	99.24
30S	<i>Acinetobacter</i>	<i>baumannii</i>	96.51

Table 4.7: Distribution of selected bacterial isolates on different food contact surfaces

Isolates	Food Items (%)					Percentage of isolates (%)
	Spoon	Fork	Table	Plate	Glass	
<i>Enterococcus</i>	1	-	-	-	1	2 (8.33)
<i>Enterobacter</i>	2	3	-	2	1	8 (33.33)
<i>Bacillus</i>	-	-	1	-	-	1 (4.17)
<i>Klebsiella</i>	1	-	2	-	-	3 (12.5)
<i>Staphylococcus</i>	-	1	1	1	-	3 (12.5)
<i>Exiguobacterium</i>	-	1	-	1	1	3 (12.5)
<i>Aeromonas</i>	-	1	1	-	-	2 (8.33)
<i>Alcaligenes</i>	-	1	-	-	-	1 (4.17)
<i>Acinetobacter</i>	1	-	-	-	-	1 (4.17)
Total out of 24 (%)	20.83	29.17	20.83	16.67	12.5	

4.1.5 Result of Antibiotic Susceptibility Pattern

The Clinical and Laboratory Standards Institute (CLSI) guideline was used to assess the sensitivity pattern of the isolated bacteria against the antibiotics that are frequently prescribed in the study (Almansour et al., 2023). The agar disc diffusion method was employed to assess the antibiotic susceptibility of pathogenic bacteria to six commonly

prescribed and available antibiotics: ciprofloxacin, amikacin, kanamycin, imipenem, meropenem, and ceftazidime. Table 4.8 displays the multiple antibiotic resistance indexes. From the table, 34.78% isolates (24/69) were resistant to multiple antibiotics, and the isolates were categorized into 16 patterns.

Table 4.8: The patterns of antibiotic resistance profiles and multiple antibiotic resistance (MAR) indexes of 24 bacterial isolates

Pattern	Strain No.	Antibiotic Resistant Profiles	MAR Index	Isolate No./Total isolates (% of occurrence)
I	6G	CIP10AK30K30MEM10CAZ30	0.833	1/24 (0.0417)
II	20S	AK30K30IPM10MEM10CAZ30	0.833	1/24 (0.0417)
III	21F	CIP10IPM10MEM10CAZ30	0.667	1/24 (0.0417)
IV	22F	CIP10K30MEM10CAZ30	0.667	1/24 (0.0417)
V	25S	AK30K30MEM10	0.5	1/24 (0.0417)
VI	26P, 27F, 27P, 27T	CIP10AK30K30	0.5	4/24 (0.1667)
VII	20G	CIP10IPM10MEM10	0.5	1/24 (0.0417)
VIII	22S	K30MEM10CAZ30	0.5	1/24 (0.0417)
IX	12F, 18S, 22G, 24F, 28T	AK30MEM10	0.333	5/24 (0.2083)
X	26G	AK30IPM10	0.333	1/24 (0.0417)
XI	25T	AK30CAZ30	0.333	1/24 (0.0417)
XII	22P, 30S	MEM10CAZ30	0.333	2/24 (0.0833)
XIII	20F	IPM10MEM10	0.333	1/24 (0.0417)
XIV	19T	IPM10CAZ30	0.333	1/24 (0.0417)
XV	26F	CIP10K30	0.333	1/24 (0.0417)
XVI	7T	K30CAZ30	0.333	1/24 (0.0417)

In Pattern I, there was a single isolate (6G) with a MAR index of 0.833. The strain was found to be resistant to ceftazidime, meropenem, kanamycin, amikacin, and ciprofloxacin. Meanwhile, Pattern II (20S) had a MAR index of 0.833 and was resistant to amikacin, ceftazidime, imipenem, meropenem, and imipenem. The MAR index for Pattern

III (21F) was 0.667 and was resistant to ceftazidime, ciprofloxacin, imipenem, and meropenem. On the other hand, pattern IV (22F) with a MAR value of 0.667 was resistant to ciprofloxacin, ceftazidime, kanamycin, and meropenem. The following pattern, V (25S), had a MAR index 0.5 and was resistant to kanamycin, amikacin, and meropenem. The ciprofloxacin, amikacin, and kanamycin-resistant Pattern VI (26P, 27F, 27P, and 27T) had a MAR index 0.5. With a MAR value of 0.5, Pattern VII (20G) exhibited resistance to imipenem, meropenem, and ciprofloxacin. Pattern VIII (22S) with a MAR index 0.5 showed resistance to ceftazidime, kanamycin, and meropenem. Patterns IX–XVI had a MAR score of 0.333 and showed antibiotic resistance. Pattern IX (12F, 18S, 22G, 24F, and 28T) exhibited resistance to amikacin and meropenem; Pattern X (26G) demonstrated resistance to amikacin and imipenem; Pattern XI (25T) showed resistance to amikacin and ceftazidime; Pattern XII (22P and 30S) was resistant to meropenem and ceftazidime; Pattern XIII (20F) displayed resistance to imipenem and meropenem; Pattern XIV (19T) was resistant to imipenem and ceftazidime; Pattern XV (26F) exhibited resistance to ciprofloxacin and kanamycin; and finally, Pattern XVI (7T) was resistant to kanamycin and ceftazidime.

The result in Table 4.9 indicated that *Enterococcus* spp. exhibited the highest susceptibility to kanamycin, meropenem, and ceftazidime (100%), followed by ciprofloxacin and amikacin (50%). For *Enterobacter* spp., antibiotic susceptibility was highest for amikacin (87.5%), followed by kanamycin (62.5%), meropenem (50%), ciprofloxacin and imipenem (37.5%), and ceftazidime (12.5%). *Bacillus* spp. exhibited resistance solely to two antibiotics: kanamycin and ceftazidime. For *Klebsiella* spp., antibiotic susceptibility was highest for amikacin (100%), meropenem (66.67%), and both kanamycin and ceftazidime (33.33%). The antibiotic susceptibility of *Staphylococcus* spp. was highest for imipenem (100%), followed by ciprofloxacin, meropenem, and ceftazidime

(66.67%). The antibiotic susceptibility of *Exiguobacterium* spp. was highest for meropenem (100%), followed by ceftazidime (66.67%) and ciprofloxacin, amikacin, and kanamycin (33.33% each). *Aeromonas* spp. exhibited the highest antibiotic susceptibility to amikacin (100%), followed by ciprofloxacin, kanamycin, and meropenem (50%). Ciprofloxacin and kanamycin were the sole antibiotics that *Alcaligenes* spp. were resistant to. Lastly, *Acinetobacter* spp. was only resistant to two antibiotics: ceftazidime and meropenem.

Table 4.9: Antibiotic susceptibility patterns of bacterial isolates

Types of antibiotics (Sensitive/ Resistant)		Number of isolates (%)								
		<i>Enterobacter</i>	<i>Enterococcus</i>	<i>Bacillus</i>	<i>Staphylococcus</i>	<i>Klebsiella</i>	<i>Exiguobacterium</i>	<i>Aeromonas</i>	<i>Acinetobacter</i>	<i>Alcaligenes</i>
Ciprofloxacin	S	3 (37.5)	1 (50)	-	2 (66.67)	-	1 (33.33)	1 (50)	-	1 (100)
	R	5 (62.5)	1 (50)	-	1 (33.33)	-	2 (66.67)	1 (50)	-	0 (0)
Amikacin	S	7 (87.5)	1 (50)	-	-	3 (100)	1 (33.33)	2 (100)	-	-
	R	1 (12.5)	1 (50)	-	-	0 (0)	2 (66.67)	0 (0)	-	-
Kanamycin	S	5 (62.5)	2 (100)	1 (100)	-	1 (33.33)	1 (33.33)	1 (50)	-	1 (100)
	R	3 (37.5)	0 (0)	0 (0)	-	2 (66.67)	2 (66.67)	1 (50)	-	0 (0)
Imipenem	S	3 (37.5)	-	-	3 (100)	-	-	-	-	-
	R	5 (62.5)	-	-	0 (0)	-	-	-	-	-
Meropenem	S	4 (50)	2 (100)	-	2 (66.67)	2 (66.67)	3 (100)	1 (50)	1 (100)	-
	R	4 (50)	0 (0)	-	1 (33.33)	1 (33.33)	0 (0)	1 (50)	0 (0)	-
Ceftazidime	S	1 (12.5)	2 (100)	1 (100)	2 (66.67)	1 (33.33)	2 (66.67)	-	1 (100)	-
	R	7 (87.5)	0 (0)	0 (0)	1 (33.33)	2 (66.67)	1 (33.33)	-	0 (0)	-

4.1.6 Food Safety Management System Implementation and Hazard Analysis and Critical Control Point (HACCP) Documentation in Selected P2 Category Food Establishments

Table 4.10 shows the adoption of food Safety management systems among P2 category food establishments in Kuching and Kota Samarahan. There is a significant distinction between Kota Samarahan and Kuching with respect to the implementation of HACCP. The HACCP plan is documented in only 2 out of 14 food outlets in Kuching, which is approximately 14.3%. The remaining 12 outlets rely solely on Food Safety is the Responsibility of the Industry (MeSTI) and Clean, Safe, and Healthy (BeSS) without HACCP certification. In contrast, none of the 16 food outlets in Kota Samarahan have implemented HACCP, resulting in a 0% HACCP compliance rate. This implies that formalized food safety systems are more established in Kuching, which is likely the result of a higher concentration of larger, export-oriented, or regulated establishments that are subject to stricter market or legal compliance.

Both Kuching and Kota Samarahan exhibit absolute adherence to the MeSTI and BeSS programs when examining their baseline food safety initiatives. All locations in both regions regularly follow these standards, which means that food hygiene meets the minimal legal and regulatory requirements. Still, the fact that Kuching has published HACCP plans shows that it has more formalization, traceability, and preventative control than Samarahan, where food safety standards are mostly not recorded and are very basic. The difference in how HACCP is used in the two places affects how well food safety works. HACCP plans written down at Kuching outlets make it easier for them to keep track of essential control points, stop cross-contamination, and keep organized records. Even though MeSTI and

BeSS requirements are followed, the lack of HACCP-certified stores in Samarahan may mean food safety violations are more likely to happen. Kuching has demonstrated early adoption of advanced food safety management systems, which indicates a trend toward higher-quality food safety practices. Kota Samarahan, on the other hand, may benefit from targeted training, capacity building, and incentives to implement HACCP. In general, both districts meet baseline food safety requirements.

Table 4.10: Status of Documented HACCP Plans in P2 Category Food Establishments in Kuching and Kota Samarahan

Location of Premises	Food Outlet Identification	Type of Food Safety Management System	Documented HACCP Plan
Kuching	2, 14	HACCP, MeSTI, BeSS	Yes
	1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13	MeSTI, BeSS	No
Kota Samarahan	15–30	MeSTI, BeSS	No

4.1.7 Result of Hazard Analysis of P2 Category Food Establishments in Kuching and Kota Samarahan

The hazards identified in the Hazard Analysis and Critical Control Point (HACCP) analysis were divided into two categories: hazards associated with raw materials and hazards associated with process steps. This analysis included a comprehensive summary of all potential hazards and any measures to mitigate identified hazards that were associated with each raw material (refer to Table 4.11) and process steps (refer to Table 4.12).

All raw meat materials (raw chicken, beef, seafood, and poultry) were identified as critical control points (CCPs) except for non-raw meat materials such as rice, vegetables, and seasonings. The primary concern with the receipt of raw beef and chicken meat was

the potential presence and proliferation of enteric pathogens (biological hazards) such as *Salmonella* spp., *Campylobacter* spp., *E. coli*, and *Listeria monocytogenes*, which could induce foodborne disease outbreaks (Wiatrowski et al., 2023; Almansour et al., 2023). *Campylobacter* spp., *Salmonella* spp., and *E. coli* were the pathogens associated with poultry. Similarly, *Vibrio* spp., *Salmonella* spp., and *Shigella* spp. were the foodborne pathogens associated with raw seafood. The prevention control and measures that could have been implemented, as per the HACCP analysis in Table 4.11, were as follows: all raw materials must be procured from reputable suppliers in accordance with the agreed-upon specifications. Incoming materials were verified against the established acceptance criteria upon their receipt. Organoleptic examinations (appearance, scent, any defects or abnormalities) were conducted to detect any physical damage to the raw material, as well as evidence of rodent, insect, or filthy matters. Additionally, certifications regarding food safety, date of receipt, product name, batch number, quantity and weight received in comparison to the document, and supplier name would be assessed (Hasnan et al., 2022). The biological hazard would be mitigated by a subsequent phase that involves the application of appropriate thermal treatment (refrigerated storage). Nevertheless, any raw materials that do not satisfy the necessary specifications would be returned to the supplier (Hasnan et al., 2022).

In the hazard analysis for food steps (Table 4.12), three CCPs were identified: biological hazards associated with refrigerated storage of products (chilling), cooking, and reheating the food. The primary concern regarding refrigerated storage of products was the potential development of foodborne pathogens or spores, such as *Listeria monocytogenes* if the storage temperature exceeded the temperature danger zone for an extended period. Consequently, food handlers who touched contaminated food or surfaces and subsequently

relocated to other areas without washing their hands or changing their gloves or apparel could transmit pathogens throughout the facility. This could result in the extensive contamination of products and, in the end, the occurrence of foodborne illness outbreaks. According to the HACCP analysis, this CCP could be managed by maintaining the cold room and all products within the 5 °C danger zone, implementing a first-in, first-out (FIFO) system to guarantee the utilization of items before spoilage, and ensuring that employees adhere to stringent hygiene and training protocols (Hasnan et al., 2022).

Next is the biological threat of foodborne pathogens such as *Enterococcus* spp., *Bacillus* spp., and *Enterobacter* spp. that emerged as a big concern during cooking. Not heating it to a high enough temperature allowed bacteria from the ingredients, the workers, or the surroundings to remain. With a food thermometer checking that the item reached the proper internal temperature and careful monitoring of cooking time, the HACCP analysis suggested that this could be handled. Finally, the primary worry throughout the reheating process was the biological danger presented by foodborne pathogens (such as *Bacillus* spp. and *Staphylococcus* spp.). This occurred because bacteria might not be adequately eradicated due to insufficient heating, a typical issue when reheating food in large quantities, ultimately resulting in foodborne illness outbreaks. Thus, according to the HACCP analysis, this could be managed or averted by employing a food thermometer to verify that food attained a safe internal temperature while cooking and reheating, while meticulously monitoring both the temperature and reheating duration.

The HACCP analysis conducted in this study is a general hazard identification for P2 category food establishments, rather than a comprehensive HACCP plan for each individual outlet. This is a critical point to emphasise. Focus was placed on the identification

of critical control points and common potential hazards that were observed throughout the P2 premises. Although this section offers a comprehensive summary of the category's primary risks and control areas, it is important to note that the results should not be used as a replacement for a comprehensive site-specific HACCP plan. This plan would necessitate detailed process mapping, validation, and continuous monitoring that are customised to each establishment.

Table 4.11: Hazard analysis worksheet for raw material/ packaging material in P2 category food establishments

Raw material/ Packaging material	Potential hazard state (biological, chemical, physical)	Justification for inclusion/exclusion as a hazard	Is this a significant hazard?				Prevention and control measures	Is this raw material/ packaging material a sensitive material?			
			Seve- rity	Like- lihood	Signi- ficant	Yes/No		Q 1	Q 2	Q 3	Yes/No
Raw non-meat material (rice, vegetables, spices, etc)	B: Presence of <i>Bacillus cereus</i> (rice), <i>E. coli</i> and <i>Salmonella</i> spp. on vegetables	Enteric pathogens associated with foodborne disease outbreak	3	B	9	Yes	1.Obtaining the most recent certifications and reports from suppliers about food safety reports and microbiology tests 2. Random sampling for internal quality control and annual audits of suppliers 3. Unapproved components are returned to their original sources 4. The hazard will be mitigated by a subsequent CCP via appropriate thermal treatment. 5. Implementing dry storage prerequisite program (FIFO system)	/	/	-	No

Table 4.11 continued

	C: Fertilizer chemical (vegetables)	All vegetables are organically certified	4	D	21	No	1. Visual inspection for quality and appearance 2. Random sampling for additional quality control 3. Supplier return of non-conform ingredients	-	-	-	No
	P: Pest, filthy and foreign matters (all ingredients)	Risk is minimal because of visual checks for package integrity and random sampling for ingredients inspection.	4	C	18	No	Visual inspection for quality and appearance, random sampling for additional quality control, and supplier return of non-conform ingredients	-	-	-	No
Poultry	B: Enteric pathogens such as <i>Campylobacter</i> spp., <i>Salmonella</i> spp., <i>E. coli</i>	Enteric pathogens associated with foodborne disease outbreak	2	B	5	Yes	1. Obtaining the most recent certifications and reports from suppliers about food safety reports and microbiology tests 2. Random sampling for internal quality control and annual audits of suppliers 3. Unapproved components are returned to their original sources 4. The hazard will be mitigated by a subsequent CCP via appropriate thermal treatment.	/	/	/	Yes

Table 4.11 continued

	C: Antibiotic, hormones	Induce and accelerate antibiotic resistance development	2	C	8	Yes	1. Review supplier and manufacturer records and certificates of analysis to confirm antibiotic composition is acceptable. 2.Periodic supplier inspection.	-	-	-	No
	P: Pest, filthy and foreign matters (all ingredients)	Risk is minimal because of visual checks for package integrity and random sampling for ingredients inspection.	4	C	18	No	1.Visual inspection. 2.Use metal or solid detector to detect foreign materials.	-	-	-	No
Raw chicken/ beef meat	B: Enteric pathogens such as <i>Salmonella</i> spp., <i>Campylobacter</i> spp., <i>E. coli</i> , <i>Listeria monocytogenes</i>	Enteric pathogens associated with foodborne disease outbreak	2	B	5	Yes	1. Ensure product is received in frozen condition 2.Obtaining the most recent certifications and reports from suppliers about food safety reports and microbiology tests 3. Unapproved components are returned to their original sources 4. The hazard will be mitigated by a subsequent CCP via appropriate thermal treatment.	/	/	/	Yes

Table 4.11 continued

	C: Veterinary drug residue (antibiotic)	Induce and accelerate antibiotic resistance development	2	C	8	Yes	1. Review supplier and manufacturer records and certificates of analysis to confirm antibiotic composition is acceptable. 2.Periodic supplier inspection.	-	-	-	No
	P: Metal fragments from cutting blade	May cause internal injury	4	D	21	No	1.Visual inspection. 2.Use metal or solid detector to detect foreign materials.	-	-	-	No
Raw Seafood	B: Enteric pathogens such as <i>Aeromonas</i> spp., <i>Enterobacter</i> spp., <i>Acinetobacter</i> spp., <i>Vibrio</i> spp., <i>Salmonella</i> spp., <i>Shigella</i> spp.	Enteric pathogens associated with foodborne disease outbreak	2	B	5	Yes	1. Ensure product is received in frozen condition 2.Obtaining the most recent certifications and reports from suppliers about food safety reports and microbiology tests 3. Unapproved components are returned to their original sources 4. The hazard will be mitigated by a subsequent CCP via appropriate thermal treatment	/	/	/	Yes

Table 4.11 continued

	C: Heavy metals like mercury. Contamination from pesticides or industrial chemicals used in the fishing environment.	Toxic and may be lethal to human health in a long term	2	C	8	Yes	1.Ensure seafood is from a reputable supplier, temperature is maintained during transport and implement Good Manufacturing Practices and Good Hygiene Practices. 2.Chemicals are approved for food use and used according to manufacturer's instructions.	-	-	-	No
	P: Fish bones, metal fragments, or other foreign objects	Choking hazard, cause internal injury	4	C	18	No	1.Visual inspection. 2. Use metal or solid detector to detect foreign materials.	-	-	-	No
Packaging	B: Plastic packaging comes into contact with surfaces or equipment that are contaminated with harmful pathogens	1.Microorganisms in packing materials can migrate to food during packaging. 2.Packaging can introduce microorganisms from contaminated surfaces, equipment, or personnel to food.	3	C	13	No	1.Regularly sanitize all surfaces and equipment that come into contact with packaging materials and products. 2.Implement strict hygiene protocols for employees, including regular handwashing, wearing clean clothing, and avoiding cross-contamination between different areas of the facility,	-	-	-	No

Table 4.11 continued

	C: Some plastic materials used for packaging may contain chemicals such as BPA	Bisphenol A (BPA) is associated with a variety of health issues including developmental and hormonal problems	2	C	8	Yes	1.Choose packaging materials that are safe for food contact especially labelled "BPA-free" 2.Manufacturers comply with relevant regulations of food packaging	-	-	-	No
	P: Metal fragments, certain plastics or glass shards	May cause internal injury	4	C	18	No	1.Use packaging materials suitable for the product and ensure proper sealing to prevent contamination during storage and transport. 2.Visual inspection	-	-	-	No

Table 4.12: Hazard analysis worksheet for process step

Raw material/ Packaging material	Potential hazard state (biological, chemical, physical)	Justification for inclusion/exclusion as a hazard	Is this a significant hazard?				What measures can be applied to control this significant hazard?	Is this process step a Critical Control Point?				
			Seve- rity	Like- lihood	Signi- ficant	Yes/No		Q 1	Q 2	Q 3	Q 4	Yes/ No
Purchase	B: Enteric pathogens such as <i>Enterococcus</i> spp., <i>Staphylococcus</i> spp., <i>Enterobacter</i> spp., <i>Alcaligenes</i> spp.	Enteric pathogens associated with foodborne disease outbreak	2	B	5	Yes	1. Purchase food from trusted sources that prioritize food safety. 2. Store purchased products properly by keeping perishable foods refrigerated or frozen soon after purchase. 3. A visual inspection of packing integrity and random sample for ingredient analysis render hazards unlikely to occur. 4. Unapproved ingredients are returned to their original sources	/	/	-	-	No

Table 4.12 continued

	C: Raw meat (antibiotics, hormone), vegetables (herbicides)	1. Promote antibiotic resistance. 2. Can cause long-term acute and chronic health issues.	2	C	8	Yes	1. Review supplier and manufacturer records and certificates of analysis to confirm antibiotic composition is acceptable. 2. Select reliable food sources to ensure food safety. 3. Consider environmental contamination, especially fresh products and seafood.	/	/	-	-	No
	P: Glass shards, metal fragments, insects or dirt	Risk is minimal because of visual checks for package integrity and random sampling for ingredients inspection.	4	D	21	No	Visual inspection for quality and appearance, random sampling for additional quality control, and supplier return of non-conform ingredients	/	/	-	-	No
Refrigerated storage of products (chilling)	B: Enteric pathogens such as <i>Listeria monocytogenes</i> spp., <i>Bacillus</i> spp., <i>Enterobacter</i> spp.	The pathogen or spores may develop if the storage temperature exceeds the temperature danger zone for an extended period	2	D	12	No	1. The temperature of the cold room and products is meticulously regulated to prevent exceeding the danger zone threshold of -18 °C. 2. FIFO (first in, first out) system to guarantee all products are dispensed prior to their expiration date. 3. Staff hygiene and training	/	/	/	/	Yes
	C: None	-	-	-	-	-	-	-	-	-	-	-

Table 4.12 continued

	P: None	-	-	-	-	-	-	-	-	-	-	-
Preparation	B: Enteric pathogens such as <i>Enterococcus</i> spp., <i>Bacillus</i> spp., <i>Enterobacter</i> spp., <i>Alcaligenes</i> spp.	All the ingredients have been controlled earlier at receipt (purchase step)	2	B	5	Yes	1.Clean surfaces and use separate cutting boards and utensils for raw and cooked foods to avoid cross-contamination. 2.Cleaning hands with soap and water before and after cooking is important for food handlers.	/	/	-	-	No
	C: Cleaning/sanitizing chemical residues	Compliance with cleaning and sanitising standard operating procedures	5	D	24	No	1. Chemical residues are unlikely to be present when cleaning SOPs are available.	/	/	-	-	No
	P: Pest hairs or faeces	Pest infestation	4	C	18	No	1. Availability of pest barrier and management system 2.Set up regular storage and equipment cleaning and maintenance.	/	/	-	-	No
Cooking	B: Enteric pathogens (<i>Enterococcus</i> spp., <i>Bacillus</i> spp., <i>Enterobacter</i> spp.)	Pathogens that originate from ingredients, personnel, or the environment may not be eliminated	1	B	2	Yes	1. Use a food thermometer to ensure that food is cooked to the proper internal temperature. 2. The temperature and cooking time are rigorously monitored.	/	/	/	/	Yes

Table 4.12 continued

	C: None	-	-	-	-	-	-	-	-	-	-	-
	P: Hair, fingernails, jewellery, faeces of pests	Can cause choking/injury, and harbour bacteria, increasing foodborne illness risk.	4	C	18	No	1. Implement proper food handling practices. 2. Regular maintenance to repair or replace defective equipment. 3. Use an effective pest management plan.	/	/	-	-	No
Cooling	B: Enteric pathogens such as <i>Enterococcus</i> spp., <i>Bacillus</i> spp., <i>Enterobacter</i> spp., <i>Alcaligenes</i> spp.	Survival of enteric pathogens that can cause foodborne disease outbreaks	2	B	5	Yes	1. The temperature and cooling time are rigorously monitored.	/	/	-	-	No
	C: None	-	-	-	-	-	-	-	-	-	-	-
	P: Hair, fingernails, glass, jewellery, paint flakes, dust	Can cause choking or injury, and harbour bacteria, increasing foodborne illness risk.	5	D	24	No	1. Regularly inspect equipment for damage or contamination and clean it thoroughly before use. 2. Implement proper food handling practices to ensure staff wear hair restraints and avoid wearing jewellery.	/	/	-	-	No

Table 4.12 continued

Hot-holding	B: Enteric pathogens such as <i>Enterococcus</i> spp., <i>Bacillus</i> spp., <i>Enterobacter</i> spp., <i>Alcaligenes</i> spp.	Survival of enteric pathogens that can cause foodborne disease outbreaks	2	B	5	Yes	1.Keep food hot or above 60°C. 2. The temperature and hold-holding time are rigorously monitored.	/	/	-	-	No
	C: None	-	-	-	-	-	-	-	-	-	-	-
	P: Pest hairs or faeces	Pest infestation	4	C	18	No	1. Availability of pest barrier and management system 2.Set up regular storage and equipment cleaning and maintenance.	/	/	-	-	No
	B: Enteric pathogens such as <i>Bacillus</i> spp., <i>Staphylococcus</i> spp.	Pathogens may not be sufficiently eliminated due to inadequate heating, common to happen when reheating in large batches	1	B	2	Yes	1.Use a food thermometer to ensure food reaches a safe internal temperature during cooking and reheating. 2. The temperature and reheating time are strictly monitored.	/	/	/	/	Yes
	C: None	-	-	-	-	-	-	-	-	-	-	-

Table 4.12 continued

	P: Hair, fingernails, glass, jewellery, paint flakes, dirt	Can cause choking/injury, and harbour bacteria, increasing foodborne illness risk.	5	D	24	No	1.Regularly inspect equipment for damage or contamination and clean it thoroughly before use. 2.Proper food handling practices to ensure staff wear hair restraints and avoid wearing jewellery	/	/	-	-	No
	B: Enteric pathogens such as <i>Enterococcus</i> spp., <i>Staphylococcus</i> spp., <i>Enterobacter</i> spp.	Pathogen growth due to time-temperature abuse	2	B	5	Yes	1.Regularly clean and disinfect food preparation surfaces and equipment. 2. Clean and sterilize utensils between uses or use different ones for raw and cooked foods. 3. The food temperature is strictly monitored before serving	/	/	-	-	No
	C: Cleaning/ sanitizing chemical residues	Compliance with cleaning and sanitising standard operating procedures	5	D	24	No	1. Chemical residues are unlikely to be present when cleaning SOPs are available.	/	/	-	-	No
	P: Pest hairs or faeces	Pest infestation	4	C	18	No	1. Availability of pest barrier and management system 2. Set up regular storage and equipment cleaning and maintenance.	/	/	-	-	No

4.2 Discussion

The MPN technique was implemented in the current study, which revealed a contamination prevalence of 46% in 150 food contact surface samples from P2 category establishments in Kuching and Kota Samarahan. Certain outlets exhibited extremely high microbial loads exceeding 1,100 MPN/mL, while the lowest microbial loads at 3.0 MPN/mL and contamination was not uniformly distributed among food contact surfaces. Although there were disparities in surface type and magnitude, these results indicate a contamination profile that is heterogeneous and consistent with previous research conducted in Malaysia. In Kuching Ramadan bazaars, for example, high aerobic plate counts and frequent detection of *Bacillus cereus* and *Escherichia coli* in ready-to-eat foods were reported, suggesting that contamination is not limited to utensils but also affects served products (Azmi et al., 2023; Mohamad et al., 2024). Although bazaar studies concentrated on ready-to-eat food items, the current findings corroborate that utensils and tables are one of the critical points of contact between food and handler that serve as substantial reservoirs for microbial persistence in Sarawak food premises.

In Peninsular Malaysia, the prevalence of bacterial contamination was observed in accordance with utensil-based studies conducted in the cafeteria environments in Klang Valley. The presence of *E. coli* on spoons in restaurants was observed by Nahar and Mahyudin (2018), while Zulfakar et al. (2018) discovered elevated bacterial loads on cutting boards and implements in university cafeterias. The findings of these studies, which did not employ the MPN-PCR, are in agreement with the current observation that spoons, forks, and tables exhibited the maximum degree of contamination. Utensil cleansing and sanitation practices continue to be a systemic vulnerability in Malaysian food premises, as evidenced

by the observed similarity among regions. While variation in absolute contamination values may be attributed to discrepancies in detection metrics (MPN/mL) in the present study versus colony forming unit (CFU) counts in other studies, the consistent identification of utensils as focus highlights a shared sanitation challenge across different contexts.

Outlets 7, 20, 22, 24, 26, and 27 exhibited extraordinarily high MPN values ($>1,100$ MPN/mL on all sampled surfaces), which are indicative of localized hygiene management deficiencies. This was in stark contrast to outlets 2 and 14, where no contamination was detected. Differing levels of adherence to food safety management systems may account for this discrepancy. The present study determined that outlets 2 and 14 (located in Kuching) were the sole establishments with documented HACCP plans, while none of the outlets in Kota Samarahan had HACCP certification. This observation is consistent with prior research that suggests that HACCP adoption is more prevalent in larger or export-oriented premises in Peninsular Malaysia, but it is still rare among smaller establishments, notably in East Malaysia (Dima et al., 2024). Formalized food safety systems are effective in reducing microbial risks, as evidenced by the stronger contamination control in HACCP-documented outlets, even when baseline hygiene schemes such as MeSTI and BeSS are in place.

It is essential to compare the results to internationally recognized hygiene standards when determining the severity of contamination. Regulations such as the Codex Alimentarius, the hygiene laws of the European Union (EC No. 852/2004; EC No. 2073/2005), and the FDA Food Code 2023 (European Commission, 2005; Codex Alimentarius, 2023; FDA Food Code, 2023) require that all surfaces that come into contact with food be kept in an obviously clean and sanitary state and, if needed, disinfected. Conversely, surface hygiene microbiological thresholds are not specified by these

frameworks. Instead, they emphasized implementing HACCP, process control, and outcome-based verification to keep conditions clean. Similar measures are implemented in Malaysia, where establishments serving food must keep all surfaces and utensils in a "clean and sanitary condition" as stated in Food Act 1983 and the Food Hygiene Regulations 2009. However, these regulations do not specify how many microbes on surfaces that come into contact with food are acceptable. National certification programs such as MeSTI and BeSS also emphasize infrastructure, hygiene standards, and staff training rather than quantitative microbiological testing of surfaces. Hence, no MPN-based approved permissible range for food-contact surfaces exists in Malaysia.

Recent empirical benchmarks derived from scientific studies are frequently employed by researchers to assess microbial contamination on food-contact surfaces in the absence of numeric national standards. For example, hygiene assessments in food truck environments suggest that the total viable counts (TVC) on sanitized food-contact surfaces should not exceed $3.0 \log_{10}$ CFU per 100 cm^2 (approximately 30 CFU/cm²). Meanwhile, Wiatrowski et al. (2023) state that any presence of *E. coli*, *Enterobacteriaceae*, *Salmonella* spp., or *Listeria monocytogenes* is considered objectionable. Similar to this, in meat and dairy processing facilities, post-sanitation counts of generic *E. coli* should not exceed $2.0 \log_{10}$ CFU/100 cm². Values exceeding $2.4 \log_{10}$ CFU/100 cm² suggest that cleaning has failed (Abdallah-Ruiz et al., 2022). According to research conducted by Zaki et al. (2024), only 16.2% of food-contact surfaces in Egyptian university restaurant kitchens met the criteria for adequate cleanliness, which is defined as aerobic colony counts below $2.0 \log_{10}$ CFU/cm². Coliforms and potential pathogens are substantially more likely to be present on surfaces that exceeded this threshold (Zaki et al., 2024). This threshold was validated by the current result. This study consistently detected an MPN exceeding 1,100/mL, which suggests a

microbial burden that is at least one order of magnitude higher than the internationally acceptable level. The results show that the sanitation practices were not effective because the procedures for cleaning and disinfection were either not monitored enough or were structurally flawed due to factors like low sanitizer concentration, unclean water, or recontamination after cleaning. Representing a critical breach of Good Hygiene Practice (GHP) and HACCP expectations, the likelihood of pathogen transfer to ready-to-eat foods is markedly increased by the persistence of contamination at this level from a risk perspective.

A critical validation of the sanitation failures observed in Sarawak is provided by comparative evidence from a variety of global contexts. The FDA Food Code requires that food-contact surfaces in the United States remain visibly clean and effectively sanitized, with rigorous corrective procedures enforced whenever lapses occur (FDA Food Code, 2023). In general, microbial loads are maintained at a low level when sanitation is rigorously implemented; however, there is evidence that residual contamination may persist. As an illustration, a study of child-care food service surfaces discovered coliforms in nearly 25% of samples despite the implementation of sanitation protocols. This finding implies that, although routine cleaning significantly reduces contamination, it does not always achieve complete elimination, with failures frequently attributable to lapses in execution rather than systemic inadequacy (Cosby et al., 2008). Similarly, research indicates that the combination of cleaning and sanitizing significantly improves microbial reduction in comparison to cleaning alone, underscoring the significance of consistent and appropriate sanitation procedures as the foundation of contamination control (Ohman et al., 2023). In the European Union, Regulation (EC) No. 853/2004 mandates that food establishments implement HACCP-based sanitary controls, which have been demonstrated to reduce microbial

contamination on utensils and preparation surfaces, even in high-volume kitchens (European Commission, 2005). These high-compliance regions serve as evidence that sanitation verification, in conjunction with structured, mandatory food safety systems, can sustain contamination levels that are significantly lower than those observed in Sarawak.

On the other hand, the results in low and middle-income nations show how inconsistent hygiene management and weak regulatory monitoring can lead to negative outcomes. Recent research in Nigeria found that institutional facilities' food contact surfaces were heavily contaminated with *E. coli* and elevated aerobic counts. This finding suggests that there was a lack of sanitation and limited implementation of HACCP-based systems (Umaru, 2023). In Southeast Asia, contamination rates remain continuously high due to the absence of consistent microbiological surveillance. A study conducted by Schwan et al. (2020) in Cambodia during the dry season found that 41% of food-contact surfaces in informal marketplaces tested positive for *Salmonella enterica*. During this period, it was determined that approximately 23-26% of the dishes, cutting boards, utensils, and hands of the food handlers at Thai restaurants were contaminated (Tasanapak et al., 2023). The fact that these prevalence levels are 40-60% higher than global empirical norms casts doubt on the efficacy of regulatory enforcement and sanitation standards.

One-third of the resistant bacteria were *Enterobacter* spp. isolates, and 34.8% of the samples examined in the study exhibited an index of multiple antibiotic resistance (MAR) greater than 0.2. This percentage is also on the rise, in conjunction with the growing prevalence of resistant strains in food-related settings in Malaysia. In meat and fish sold in stores, multidrug-resistant *Enterobacteriaceae* and *Klebsiella* spp. were identified by Cheesman et al. (2023) and Yap et al. (2022). Azmi et al. (2023) conducted an analogous

study that verified the presence of beta-lactam-resistant *B. cereus* isolates in the commodities sold at the Kuching market. The similarities between the research indicate that resistant microorganisms are present on food-contact surfaces, utensils, raw meats, and finished foods. These surfaces are capable of supporting secondary vectors for transmission. However, Sarawakian food establishments should be concerned about the novel and previously unrecognized carbapenem-resistant isolates (with MAR indices as high as 0.833) discovered in the present study. This is for the simple reason that carbapenem resistance is more frequently observed in reservoirs that involve animals and humans than in the food-service industry (Hartantyo et al., 2020). The capacity of antimicrobial-resistant Enterobacteriaceae to persist on inert surfaces for extended periods is becoming increasingly evident, particularly in biofilms formed by surfaces that have dried out. The study by Centeleghe et al. (2023) specifically demonstrates that *Klebsiella pneumoniae* can persist in biofilm form on inert surfaces for a period of up to four weeks following routine cleansing. This state enables cells to evolve into a viable but uncultivable state (Centeleghe et al., 2023). Bacteria that produce carbapenemase, such as *Klebsiella pneumoniae* and *Enterobacter cloacae*, can be found on sinks and other frequently handled surfaces in healthcare facilities (Kim et al., 2022). This demonstrates the external transmission of resistant microbes beyond patient reservoirs (Kim et al., 2022). For instance, in Brazil, *E. coli* was identified in 73.6% of samples collected from slaughterhouse apparatus and the environment. Additionally, over 80% of these isolates were resistant to multiple drugs. This demonstrates the significance of food processing industry contexts as potential sources of antibiotic resistance (Sampaio et al., 2024). This discovery is consistent with the findings of Almansour et al. (2023), who demonstrated that surfaces that are in contact with food or the environment can harbour resistant bacteria for extended periods. Against these microbes, conventional cleaning

procedures would be exceedingly difficult, if not impossible. While the majority of international reports on resistant bacteria on surfaces pertain to hospital settings (China) or abattoir environments (Brazil), the findings in current research in Sarawak are noteworthy for their presence within retail food establishments that directly serve consumers. This suggests a risky cross-domain transmission of resistance genes from the healthcare and animal production sectors to food-service ecosystems. The persistence of carbapenem-resistant organisms in such premises indicates that food-contact surfaces may now serve as an unidentified locus of AMR dissemination, thereby enhancing the likelihood of resistant bacteria entering the human food chain through routine dining practices.

High microbial loads and resistant isolates on food-contact surfaces in Sarawak are indicative of systemic deficiencies in sanitation management, which are closely associated with deficiencies in HACCP implementation. In this investigation, it was determined that the utensils and tables with the highest contamination rates were also those that produced resistant *Enterobacter*, *Klebsiella*, and *Staphylococcus* isolates. This finding suggests that deficiencies in cleaning and sanitization not only enabled the persistence of microbial organisms but also facilitated their survival. This conclusion is predictable when compared to the HACCP adoption results: only 2 of the 14 food outlets in Kuching (14.3%) had documented HACCP plans, while none of the 16 outlets in Kota Samarahan demonstrated HACCP compliance, instead relying on baseline MeSTI and BeSS programs.

This comparative analysis between Kuching and Kota Samarahan emphasizes the importance of formalizing HACCP in order to manage contamination. Outlets in Kuching that had some level of HACCP documentation were structurally more advantageously situated to identify CCPs, document hazards, and employ preventive measures.

Contamination was still observed; however, the presence of HACCP documentation in Kuching implies that at least partial recognition of utensil hygiene and culinary steps as critical nodes requiring control has been made. On the other hand, the absence of HACCP documentation in Kota Samarahan was accompanied by a significantly greater variance in microbial burdens across outlets, as evidenced by the ANOVA results, which indicated a higher F-statistic (17.24 in Kota Samarahan vs. 2.68 in Kuching). This statistical result is compelling evidence that the absence of HACCP systems in Kota Samarahan resulted in more erratic and less controlled sanitation practices, which raised the probability of resistant organisms lingering on high-use utensils and tables.

Trends on a national and regional level are consistent with these findings. Establishments in Peninsular Malaysia that do not use HACCP or just partially use it have surfaces and utensils that are consistently contaminated. Nahar and Mahyudin (2018) conducted surveys in Klang Valley cafeterias and restaurants which found a strong correlation between the high occurrence of *E. coli* and *S. aureus* on utensils and insufficient cleaning measures. Similar research by Sabaruddin et al. (2019) revealed that food courts still have contaminated utensils and countertops even with basic hygiene certificates. These findings support the hypothesis that confirming HACCP protocols is more important for risk mitigation than ensuring baseline compliance (Sabaruddin et al., 2019). There is substantial evidence from Thailand that HACCP can help reduce the occurrence of pathogens and antibiotic resistance. Wu et al. (2019) investigated two swine slaughterhouses for the purpose of identifying *Salmonella* infection and trends in resistance. Only one of the establishments had the HACCP certification. The rate of *Salmonella* carcass contamination was 48.9% lower at a HACCP-certified facility compared to a non-HACCP location, with the former having an elevated rate of 14.3% (Wu et al., 2019). Lower rates of *Salmonella*

infection and levels of antimicrobial-resistant isolates were observed in Thailand pork slaughterhouses that were HACCP-certified compared to those that were not. These results show that the use of HACCP practices reduces the prevalence of pathogens and helps to control the spread of antibiotic resistance in the food manufacturing process (Saenkankam et al., 2025). These results were further supported by a subsequent regional analysis, which found that, in Southeast Asian facilities, HACCP certification consistently lowers pathogen prevalence and inhibits the transmission of resistant strains compared to non-certified facilities (Sirichokchatchawan et al., 2021). Viewed collectively, these comparisons emphasize the importance of the Sarawak findings: they offer local proof that chronic resistant organisms and high microbial diversity are directly associated with insufficient or non-existent HACCP documentation, as shown in Kota Samarahan.

The integration of microbiological data with HACCP evaluation is a strong suit of this study. It demonstrates the direct relationship between management strategies and contamination outcomes. This study methodically examined food-contact surfaces and utensils in two areas of Sarawak, in contrast to previous research that mostly concentrated on bazaar foods and ready-to-eat foods. It merged this information with statistics on the deployment of HACCP. The lack of contamination has long been associated with the presence and efficacy of HACCP systems according to worldwide standards for quality control. These criteria are met by this methodological integration. The finding that resistant isolates were more prevalent in facilities that did not have HACCP documentation supports the idea that HACCP is not merely a legal requirement; it is a practical component of food safety performance.

Contamination trends in Peninsular Malaysia and Sarawak are comparable, indicating systemic weaknesses in hygiene standards are still very much present. Significant control points in hazard analysis in both locations were determined to be inadequate temperature control during chilling and reheating, staff hygiene lapses, and poor utensil cleaning (Eskoai et al., 2020; Lai et al., 2024; Yang et al., 2025). The fact that these failures are happening in the same places as in other Southeast Asian countries shows that food safety management is a systemic problem, especially in areas that are not HACCP certified. New data from the Philippines has highlighted the gravity of these problems. An analysis of wet fish stands on Bohol Island showed an alarmingly high concentration of microbes on surfaces that come into contact with food. Levels exceeding the internationally permissible standards were detected on chopping boards, reaching 7.1×10^6 CFU/cm². Consistent with the high microbiological loads in Sarawak's poorly managed stores, these results highlight fundamental sanitation deficiencies in high-risk retail settings (Magallanes & Aguanza, 2024). Even though they had access to basic infrastructure like water hookups and work tables, many vendors in Jakarta, Indonesia, failed to adhere to proper hygiene and sanitation procedures. Vendors neglected to wash their utensils properly and wasted water regularly, according to the results of the interviews and observations. Despite the venues' best efforts to encourage participants to exercise good personal hygiene, a few vendors still did not (Harianto & Ardani, 2021). These results are consistent with what was shown in Sarawak, where establishments without HACCP paperwork also showed serious problems with basic sanitation, even if they had access to facilities. As seen in previous research, the failure to operationalize infrastructure through standardized food safety measures, not the location, is the cause of contamination. Water quality was an essential factor in the sanitary outcomes of street food sellers in a systematic review carried out in Thailand (Mulyodarsono &

Kristopo, 2024). Despite the implementation of surface cleansing, many vendors still used stored "bucket water" instead of potable tap water to wash their equipment and ingredients. This practice created a continuing danger of microbiological contamination. This is disturbingly similar to the cleanliness issues plaguing Malaysian restaurants. The lack of effective sanitizers and water of poor quality hampered cleaning and disinfection efforts (Mulyodarsono & Kristopo, 2024). These parallels emphasize, in particular, that microbiological contamination in food is more common in establishments that are not HACCP-certified than in geographically isolated ones.

Part of the observed disparities between this study and others can be attributed to detection approaches from a methodological perspective. The present study employs MPN-PCR, which enables a greater degree of sensitivity and specificity in the detection of foodborne bacteria than the conventional culture-based or plate count methodologies employed in previous Malaysian and Southeast Asian studies. As a consequence, the contamination prevalence and microbial load estimates may appear to be higher in this dataset than in comparable research that employs fewer sensitive methodologies. It is crucial to interpret results in the context of detection capabilities, and this methodological distinction underscores the necessity of harmonized surveillance protocols to facilitate meaningful comparisons between studies and regions. In the context of a broader global perspective, the results have substantial implications. The contamination of food-contact surfaces rarely surpasses benchmark thresholds in Europe and North America, where HACCP adoption is nearly universal and routinely audited, and resistant isolates are rarely reported outside of clinical environments. It is possible that Malaysian food premises are experiencing an emergent risk profile that is more akin to that of developing regions than to that of high-compliance countries, as evidenced by the recovery of carbapenem-resistant strains from

utensils in Sarawak. In addition, this underscores the necessity of immediate interventions that simultaneously address contamination control and AMR surveillance.

A number of interrelated factors can account for the significantly greater contamination rates observed in specific premises. First and foremost, the absence of HACCP documentation in outlets such as 7, 20, 22, 24, 26, and 27 (showed highest contamination rate of food contact surfaces) resulted in the informal monitoring of critical control points, which allowed for the undetected persistence of lapses in utensil cleansing, reheating, and chilling. The second factor is that structural constraints, including inadequate sanitation facilities, poor water quality, and a lack of access to effective sanitizers, may have contributed to the persistence of contamination. Third, staff training deficiencies likely contributed to the situation, as sanitation efforts can be neutralized by improper handling or recontamination following cleansing. This combination of factors explains why certain premises registered contamination on all sampled surfaces, while HACCP-documented outlets did not.

Several targeted adjustments are suggested in light of these findings. First and foremost, it is imperative to expand the implementation of HACCP in small and medium-sized outlets in order to guarantee the systematic surveillance of CCPs. Second, infrastructure enhancements, including the provision of clean water and access to standardized sanitation equipment, should be prioritized in high-risk premises. Third, staff training programs must prioritize the instruction of employees in safe storage practices, utensil washing, and hand hygiene, with regular refresher training. Fourth, inexpensive monitoring instruments, including adenosine triphosphate (ATP) samples and microbial rapid tests, should be implemented to confirm the efficacy of cleaning protocols. As a final

measure, regulatory authorities should incorporate AMR surveillance into food safety inspections to guarantee that resistant genotypes are monitored and managed as part of national food safety monitoring. All in all, the comparative analysis suggests that the contamination prevalence, resistant isolates, and low HACCP adoption rates observed in this study are in line with broader national and regional trends, albeit with specific distinctive characteristics. The identification of carbapenem-resistant organisms on food-contact surfaces in Sarawak indicates a rise in risk in comparison to previous reports, while the protective effect of HACCP documentation against contamination offers clear evidence for policy and practice interventions. The similarities among regions emphasize the fact that utensil hygiene and CCP management are shared vulnerabilities, while the differences underscore the significance of structural, regulatory, and methodological factors in determining contamination outcomes. In comparison to international microbiological standards, the contamination levels in numerous premises are significantly higher than the acceptable thresholds, indicating a systemic failure of sanitation practices. These results, taken together, bolster the case for the expansion of HACCP implementation, the improvement of food-handler training, the enforcement of international sanitation benchmarks, and the integration of AMR surveillance into food safety monitoring in Malaysia.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In summary, this study used three primary goals to evaluate the food safety assessment and microbiological quality of P2 food enterprises in Sarawak. First, 46% of 150 samples were contaminated using the most probable number (MPN) technique, indicating a high frequency of foodborne bacteria on food-contact surfaces. The bacterial loads ranged from 3 MPN/mL to over 1,100 MPN/mL, with spoons being the most contaminated samples. Analysis of Variance (ANOVA) verified that there was a significant difference between the outlets in Kuching and Kota Samarahan. These findings draw attention to serious flaws in sanitation procedures, demonstrating that present cleaning measures fall short of international hygiene standards and put customers at risk of contracting infections. Secondly, the antimicrobial resistance profiling demonstrated that 34.78% of the isolates were multidrug-resistant. *Enterobacter* spp. was the most prevalent genus (33.3%), followed by *Klebsiella*, *Staphylococcus*, and *Exiguobacterium*. Additionally, resistance to last-line antibiotics like carbapenems was found, highlighting the fact that surfaces in touch with food are not only potential sources of contamination but also possible conduits for the transmission of harmful resistant strains. Given the prevalence of antibiotic resistance and difficulty treating food poisoning, this finding highlights the significance of frequent food settings. Thirdly, none of the Kota Samarahan outlets documented their HACCP plans, and only two outlets in Kuching (14.3%) had HACCP documentation. However, all the establishments met the baseline requirements for Food Safety is the Responsibility of the Industry (MeSTI) and Clean, Safe, and Healthy (BeSS). Hazard analysis identified six

important control points, including raw meat handling, cooking, chilling, and reheating; nevertheless, systematic monitoring and preventative action are still insufficient because HACCP documentation is not widespread. These findings highlight the need for stronger food safety regulations, since public health risks are more likely to manifest in businesses that do not follow HACCP protocols.

Collectively, the findings of the research highlight the need for HACCP expansion in Sarawak, improved sanitation practices, and proactive monitoring of antibiotic resistance as means to protect consumer health and fortify the food safety system. There is also an emphasis on the worryingly high frequency of multidrug-resistant bacteria and the inadequate implementation of HACCP. This study is significant due to its status as one of the earliest comprehensive investigations of the prevalence of bacteria and antibiotic resistance on food-contact surfaces in P2 restaurants in Sarawak. Contrary to earlier research that primarily focused on food samples, the current study provides direct evidence that utensils and tables are critical reservoirs for pathogenic and resistant bacteria. By combining microbiological investigation with antimicrobial resistance profile and HACCP-based hazard identification, the study not only outlines the extent of the problem but also highlights possible methods to improve food safety procedures. These findings provide important information for food industry operators, regulators, and lawmakers looking to improve hygiene standards, increase the likelihood of uniform HACCP implementation, and protect the public from food poisoning and antibiotic resistance.

5.2 Recommendations

The prevalence of pathogenic bacteria and antimicrobial resistance on food-contact surfaces in P2 category food establishments has been effectively emphasized by this study;

however, it is important to recognize several limitations. The study was limited to a relatively small sample size of 150 swabs, which were collected exclusively from premises in Kuching and Kota Samarahan. As a result, the results cannot be applied to other types of culinary establishments or to regions throughout Sarawak and Malaysia. Furthermore, the results may only represent a particular moment in time due to the cross-sectional design, which does not account for possible changes in microbial contamination across time or seasons. Furthermore, the antimicrobial resistance profiling only included a handful of the most widely used antibiotics, so the results could not represent the whole resistance range. Finally, the precision and reproducibility of results could have been impacted because microbiological analyses were conducted using single sample readings instead of repeated trials. To overcome these constraints, researchers should increase the quality and reliability of data by making methodological improvements in future studies. To ensure more reliable results and less room for error, collecting and analysing microbiological data in triplicate for each sample is recommended. The results would be more representative if the sample size were increased and a larger variety of food restaurants from different districts were included. In addition, a longitudinal design might be ideal for this investigation. This would allow for a more thorough knowledge of the contamination dynamics and the ability to detect seasonal and long-term variations in microbial contamination. Expanding the antibiotic panel and using cutting-edge scientific techniques, such as whole-genome sequencing, could also shed light on the genetic diversity of bacterial isolates and their resistance mechanisms. All of these improvements would make future studies more accurate and provide a stronger foundation for studies on food safety.

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APPENDICES

Appendix A: Identity of bacteria samples based on reference sequences in BLAST results

Sample ID	Name of Species	Percentage Identity (%)	Position
6G	1. <i>Enterococcus faecalis</i> strain NBRC 100480	99.50	41-240
	2. <i>Enterococcus faecalis</i> strain ATCC 19433	99.50	
	3. <i>Enterococcus faecalis</i> strain JCM 5803	99.50	
7T	1. <i>Bacillus safensis</i> FO-36b	99.39	81-472
	2. <i>Bacillus pumilus</i> strain NBRC 12092	99.39	
	3. <i>Bacillus zhangzhouensis</i> strain MCCC 1A08372	99.39	
12F	1. <i>Enterobacter mori</i> strain YIM Hb-3	99.41	391-730
	2. <i>Enterobacter sichuanensis</i> strain WCHECL1597	99.41	
	3. <i>Enterobacter quasiroggenkampii</i> strain WCHECL1060	99.41	
18S	1. <i>Klebsiella pneumoniae</i> strain NBRC 14940	99.29	289-854
	2. <i>Klebsiella pneumoniae</i> strain DSM 30104	99.29	
	3. <i>Klebsiella pneumoniae</i> strain ATCC 13883	99.29	
19T	1. <i>Staphylococcus warneri</i> strain AW 25	100	331-1000
	2. <i>Staphylococcus pasteurii</i> strain ATCC 51129	99.55	
	3. <i>Staphylococcus lugdunensis</i> strain ATCC 43809	99.70	
20S	1. <i>Enterobacter cloacae</i> strain ATCC 13047	99.62	71-598
	2. <i>Enterobacter asburiae</i> strain JCM6051	99.62	
	3. <i>Enterobacter mori</i> strain YIM Hb-3	99.43	
20F	1. <i>Enterobacter hormaechei</i> subspp. <i>xiangfangensis</i> strain 10-17	95.10	51-540
	2. <i>Enterobacter cloacae</i> strain ATCC 13047	95.10	
	3. <i>Enterobacter quasihormaechei</i> strain WCHEs120003	95.10	
20G	1. <i>Staphylococcus microti</i> strain CCM 4903	80.37	661-1180
	2. <i>Staphylococcus rostri</i> strain ARI 262	80.37	
	3. <i>Staphylococcus muscae</i> strain MB4	80.37	
21F	1. <i>Staphylococcus saprophyticus</i> strain GTC 843	100	311-920

	2. <i>Staphylococcus edaphicus</i> strain CCM 8730 3. <i>Staphylococcus saprophyticus</i> subspp. <i>saprophyticus</i> ATCC 15305 = NCTC 7292	100 100	
22S	1. <i>Enterococcus faecium</i> strain NBRC 100486 2. <i>Enterococcus faecium</i> strain NBRC 100485 3. <i>Enterococcus thailandicus</i> strain NBRC 101867	100 100 100	162-620
22F	1. <i>Exiguobacterium indicum</i> strain HHS 31 2. <i>Exiguobacterium acetylicum</i> strain DSM 20416 3. <i>Exiguobacterium acetylicum</i> strain NBRC 12146	99.84 99.68 99.51	361-977
22P	1. <i>Exiguobacterium acetylicum</i> strain NBRC 12146 2. <i>Exiguobacterium acetylicum</i> strain DSM 20416 3. <i>Exiguobacterium indicum</i> strain NIO-1109	96.40 96.40 96.40	432-900
22G	1. <i>Exiguobacterium acetylicum</i> strain DSM 20416 2. <i>Exiguobacterium indicum</i> strain HHS 31 3. <i>Exiguobacterium acetylicum</i> strain NBRC 12146	99.71 99.71 99.43	12-360
24F	1. <i>Aeromonas veronii</i> strain JCM 7375 2. <i>Aeromonas veronii</i> bv. <i>veronii</i> strain ATCC 35624 3. <i>Aeromonas bivalvium</i> strain 868E	100 100 100	20-480
25T	1. <i>Klebsiella africana</i> strain SB5857 2. <i>Klebsiella pneumoniae</i> subspp. <i>rhinoscleromatis</i> strain R-70 3. <i>Klebsiella quasipneumoniae</i> subspp. <i>similipneumoniae</i> strain 07A044	100 100 99.68	407-720
28S	1. <i>Enterobacter cloacae</i> subspp. <i>dissolvens</i> strain LMG 2683 2. <i>Enterobacter cloacae</i> subspp. <i>dissolvens</i> strain ATCC 23373 3. <i>Enterobacter cloacae</i> strain NBRC 13535	99.86 99.58 99.58	61-780
26F	1. <i>Alcaligenes faecalis</i> strain NBRC 13111 2. <i>Alcaligenes ammonioxydans</i> strain HO-1 3. <i>Alcaligenes pakistanensis</i> strain NCCP-650	100 100 99.44	61-420
26P	1. <i>Enterobacter asburiae</i> strain JM-458 2. <i>Enterobacter cloacae</i> strain ATCC 13047 3. <i>Enterobacter sichuanensis</i> strain WCHECL1597	100 100 100	291-660
26G	1. <i>Enterobacter cloacae</i> strain ATCC 13047 2. <i>Enterobacter asburiae</i> strain JCM6051 3. <i>Enterobacter hormaechei</i> subspp. <i>xiangfangensis</i> strain 10-17	99.50 99.50 99.17	421-1070

27F	1. <i>Enterobacter sichuanensis</i> strain WCHECL1597 2. <i>Enterobacter quasiroggenkampii</i> strain WCHECL1060 3. <i>Enterobacter hormaechei</i> strain 0992-77	99.55 99.55 99.40	531-1200
27T	1. <i>Aeromonas veronii</i> strain JCM 7375 2. <i>Aeromonas veronii</i> bv. <i>veronii</i> strain ATCC 35624 3. <i>Aeromonas rivipollensis</i> strain P2G	99.53 99.53 99.37	422-1060
27P	1. <i>Enterobacter bugandensis</i> strain 247BMC 2. <i>Enterobacter hormaechei</i> subspp. <i>xiangfangensis</i> strain 10-17 3. <i>Enterobacter cloacae</i> strain ATCC 13047	100 99.81 99.81	411-940
28T	1. <i>Klebsiella quasipneumoniae</i> subspp. <i>quasipneumoniae</i> strain 01A030 2. <i>Klebsiella quasipneumoniae</i> subspp. <i>similipneumoniae</i> strain 07A044 3. <i>Klebsiella quasipneumoniae</i> subspp. <i>similipneumoniae</i> strain CW-D 3	99.24 99.24 99.24	506-1020
30S	1. <i>Acinetobacter baumannii</i> strain ATCC 19606 2. <i>Acinetobacter baumannii</i> strain DSM 30007 3. <i>Acinetobacter baumannii</i> strain JCM 6841	96.51 96.51 96.51	143-600

Appendix B: Result of zone diameter breakpoints of bacteria based on CLSI Guidelines, MAR Index and their resistance towards each antibiotic

Sample ID	Disc Code and Zone Diameter Breakpoints based on CLSI Guidelines (mm)						MAR Index	Resistance towards each antibiotic
	CIP-10	AK-30	K-30	IPM-10	MEM-10	CAZ-30		
1P	28	26	26	44	28	24	0.0	-
1T	25	16	17	22	26	22	0.167	AK
3G	24	18	18	26	24	26	0.0	-
4P	36	26	26	38	34	0	0.167	CAZ
5T	38	28	30	28	24	26	0.0	-
5P	30	26	20	34	32	8	0.167	CAZ
6G	18	0	0	24	18	0	0.833	CIP, AK, K, MEM, CAZ
7S	28	28	24	44	36	20	0.0	-
7F	32	26	28	40	24	22	0.0	-
7P	28	26	26	32	32	22	0.0	-
7T	28	20	12	32	30	0	0.333	K, CAZ
7G	22	18	18	26	24	30	0.0	-

8F	26	24	28	46	40	26	0.0	-
8T	24	16	16	20	24	24	0.167	AK
8G	24	18	18	26	20	26	0.0	-
9T	24	18	17	20	26	24	0.0	-
10T	30	22	22	26	24	24	0.0	-
11T	28	22	20	36	32	22	0.0	-
12F	24	14	16	22	18	26	0.333	AK, MEM
13T	28	20	20	22	18	20	0.167	MEM
13P	28	26	26	32	16	24	0.0	-
15S	32	22	22	25	18	28	0.167	MEM
16S	28	18	18	20	18	28	0.167	MEM
16G	23	18	18	26	16	19	0.167	MEM
17S	24	20	18	24	18	22	0.167	MEM
17G	29	19	18	23	28	23	0.0	-
18S	24	16	18	21	18	24	0.333	AK, MEM
18F	24	26	26	44	18	22	0.167	MEM
18P	30	28	28	42	22	24	0.0	-
19T	30	24	26	0	38	14	0.333	IPM, CAZ
20S	24	10	0	0	0	0	0.833	AK, K, IPM, MEM, CAZ
20F	22	26	18	0	0	20	0.333	IPM, MEM
20T	26	22	22	24	22	20	0.0	-
20P	27	22	22	28	24	27	0.0	-
20G	20	33	20	0	0	20	0.5	CIP, IPM, MEM
21S	24	20	18	22	24	22	0.0	-
21F	18	18	16	18	16	0	0.667	CIP, IPM, MEM, CAZ
22S	22	19	10	24	9	6	0.5	K, MEM, CAZ
22F	20	17	11	24	0	0	0.667	CIP, K, MEM, CAZ
22P	20	20	22	26	16	22	0.333	CAZ, MEM
22T	25	19	20	23	27	21	0.0	-
22G	24	16	18	22	12	22	0.333	AK, MEM
23F	23	19	19	20	26	27	0.0	-
23P	26	18	0	23	27	28	0.167	K

23G	26	18	19	23	28	26	0.0	-
24S	27	19	19	25	29	28	0.0	-
24F	24	16	16	22	16	26	0.333	AK, MEM
24P	22	8	16	24	26	20	0.167	AK
24T	28	18	18	20	28	22	0.0	-
24G	22	12	16	22	24	18	0.0	-
25S	24	16	12	24	0	18	0.5	AK, K, MEM
25T	26	16	16	24	26	14	0.333	AK, CAZ
25G	26	18	18	22	26	22	0.0	-
25P	26	18	16	20	26	22	0.0	-
26S	16	19	15	27	25	22	0.167	CIP
26F	12	18	7	23	25	19	0.333	CIP, K
26P	0	15	0	22	28	22	0.5	CIP, AK, K
26T	20	18	17	29	26	26	0.167	CIP
26G	24	16	16	14	24	24	0.333	AK, IPM
27S	22	12	16	40	38	28	0.167	AK
27F	20	10	12	20	28	18	0.5	CIP, AK, K
27P	18	16	0	24	26	20	0.5	CIP, AK, K
27T	20	10	12	36	32	26	0.5	CIP, AK, K
27G	26	14	14	20	26	22	0.167	AK
28T	22	15	16	23	14	24	0.333	AK, MEM
29T	24	30	22	44	32	22	0.0	-
30S	26	18	22	28	0	12	0.333	MEM, CAZ
30F	24	26	24	24	8	20	0.167	MEM
30P	26	22	24	34	14	20	0.167	MEM

Appendix C: The patterns of antibiotic resistance profiles and multiple antibiotic resistance (MAR) indexes of all positive bacterial isolates

Pattern	Strain No.	Antibiotic Resistant Profiles	MAR Index	No. of isolate/Total isolates (% of occurrence)
I	1T, 8T, 24P, 27S, 27G	AK30	0.167	5/69 (7.246)
II	12F, 18S, 22G, 24F, 28T	AK30MEM10	0.333	5/69 (7.246)

III	26G	AK30IPM10	0.333	1/69 (1.449)
IV	25T	AK30CAZ30	0.333	1/69 (1.449)
V	25S	AK30K30MEM10	0.5	1/69 (1.449)
VI	20S	AK30K30IPM10MEM10CAZ30	0.833	1/69 (1.449)
VII	13T, 15S, 16S, 16G, 17S, 18F, 30F, 30P	MEM10	0.167	9/69 (13.043)
VIII	22P, 30S	MEM10CAZ30	0.333	2/69 (2.899)
IX	20F	IPM10MEM10	0.333	1/69 (1.449)
X	19T	IPM10CAZ30	0.333	1/69 (1.449)
XI	26S, 26T	CIP10	0.167	2/69 (2.899)
XII	26F	CIP10K30	0.333	1/69 (1.449)
XII	26P, 27F, 27P, 27T	CIP10AK30K30	0.5	4/69 (5.797)
XIV	20G	CIP10IPM10MEM10	0.5	1/69 (1.449)
XV	6G	CIP10AK30K30MEM10CAZ30	0.833	1/69 (1.449)
XVI	21F	CIP10IPM10MEM10CAZ30	0.667	1/69 (1.449)
XVII	22F	CIP10K30MEM10CAZ30	0.667	1/69 (1.449)
XVIII	23P	K30	0.167	1/69 (1.449)
XIX	7T	K30CAZ30	0.333	1/69 (1.449)
XX	22S	K30MEM10CAZ30	0.5	1/69 (1.449)
XXI	4P, 5P	CAZ30	0.167	2/69 (2.899)