EFFECT OF FOOD-BORNE PATHOGENS FROM STREET-VENDED FOODS ON HEMATOLOGICAL PARAMETERS AND OXIDATIVE STRESS MARKERS OF WISTAR RATS

BY

Aisosa Emmanuel EKHATOR

LSC2103935

SUPERVISOR

Dr. (MRS) A.A. IMONI

DEPARTMENT OF MICROBIOLOGY,

FACULTY OF LIFE SCIENCES,

UNIVERSITY OF BENIN,

BENIN CITY

NOVEMBER, 2025.

EFFECT OF FOOD-BORNE PATHOGENS FROM STREET-VENDED FOODS ON HEMATOLOGICAL PARAMETERS AND OXIDATIVE STRESS MARKERS OF WISTAR RATS

BY

Aisosa Emmanuel EKHATOR

LSC2103935

A RESEARCH PROJECT TO BE SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE, B.SC (HONS) DEGREE IN MICROBIOLOGY, UNIVERSITY OF BENIN, BENIN CITY

NOVEMBER, 2025.

CERTIFICATION

This is to certify that this project work was carried out by **Aisosa Emmanuel EKHATOR** in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin city under my supervision.

Dr. (MRS)A. A. IMONI **DATE**

(Project Supervisor)

PROF. E.O. IGBINOSA **DATE**

(Head of Department)

DEDICATION

This project work is dedicated to God Almighty, who in his infinite mercy brought me this far in life and granted me success in all my work.

ACKNOWLEDGEMENTS

I would like to express my profound gratitude and appreciation to God Almighty who has sustained me throughout this time enabling me to successfully complete my studies at the University and also complete my project.

I was truly favored by God when I was assigned to my supervisor Dr. (MRS) A. A. Imoni , who selflessly aided me during my project. She is extremely kindhearted and understanding, despite her busy work schedule, she promptly attended to me always and never hesitated to corrected my errors.

I am also grateful to PROF. E.O. Igbinosa, the Head of Department, Mr. G.O Oribhabor and Mrs. O.B Agbonwaneten my course advisors, and all the staff members of the Department of Microbiology for their assistance.

I also use this opportunity to express my profound gratitude to my parents REV. and REV. Mrs. Efosa Ekhator and my siblings Grace Ekhator, Isaac Ekhator and Daniel Ekhator, who supported me morally, financially and spiritually throughout my education in the University of Benin.

I would like to appreciate my friends Ezekiel Awele, Oshiakpe Adaobi, Imuetiyan princewill, Igenegba Lateef, Nosa Loveth, Mba Joy, Mark Ohenren, George Okunade, Jennifer oghale, Tagbo Asher and Be Guided for their encouragement and support throughout my academic journey.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Street-vended foods provide affordable nutrition for many urban populations but often serve as vehicles for food-borne pathogens capable of causing systemic health effects. This study investigated the effects of pathogens isolated from street-vended foods on the hematological parameters and oxidative stress markers of Wistar rats. Bacteria isolated from street-vended foods were obtained for this study and identified using molecular (polymerase chain reaction) technique. Thereafter, isolates were screened for phenotypic virulence characteristics (biofilm formation, haemolysin and gelatinase production) using standard techniques. Twenty-five wistar rats weighing 169.40g-175.01g were used. Enumeration and isolation of feed samples was done using serial dilution and pour plate techniques. After one week acclimatization, rats were randomly selected into five equal groups (control, W_1,W_2, X_1 and X_2). Rats were experimentally infected with different concentrations (0.06ml and 0.1ml) of *Escherichia coli* (PX395408) and *Klebsiella pneumoniae* (PX395409). After infection, changes in the weight of rats were determined weekly. Following sacrifice, blood and tissue samples were obtained for hematology and histopathological examination. The results revealed that *E. coli* exhibited β-hemolytic activity, was positive for biofilm formation, and negative for gelatinase production, while *K. pnuemoniae* showed γ-hemolytic activity and was negative for both biofilm and gelatinase production. The total heterotrophic bacterial counts of feed samples ranged from 5.35±0.07 to 6.90±1.56×10^5 cfu/g, with isolates including *Citrobacter* spp and *Klebsiella* spp. The initial body weight of rats ranged from 169.00 ± 13.90 g to 175.00 ± 10.40 g, while the control group weighed 170.61 ± 4.90 g. After infection, the control rats gained weight steadily (189.35 ± 12.52 g), whereas infected rats showed weight reduction, ranging from 125.76 ± 11.95 g to 145.02 ± 28.94 g, indicating systemic infection. Hematological analysis revealed that the control rats maintained normal values except for slightly higher white blood cell counts compared to infected groups. Rats infected with *E. coli* (0.06 mL) recorded higher red blood cell (RBC) and hemoglobin (HGB) levels than the control, while platelet (PLT) counts significantly increased in *K. pnuemoniae*-infected rats, particularly in the high-dose group (1315±546). Significant differences ($p<0.05$) were observed in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values, while other parameters showed no significant differences ($p>0.05$). Oxidative stress markers revealed that *K. pnuemoniae* infected rats exhibited elevated superoxide dismutase (SOD) and malondialdehyde (MDA) levels, indicating oxidative damage, whereas catalase (CAT) and glutathione peroxidase (GPx) activities were significantly increased in all infected groups. These findings demonstrate that *E. coli* and *K. pnuemoniae* from street-vended foods can induce hematological alterations, oxidative stress, and weight loss in Wistar rats, underscoring the need for improved food hygiene and stricter safety regulations.

CHAPTER ONE

INTRODUCTION

Street vended foods represent a vibrant and complex component of urban food systems worldwide, serving as both a critical source of affordable nutrition for millions and a cultural expression of local culinary traditions. These informal food enterprises, characterized by their mobility and operation in public spaces, contribute significantly to urban economies while simultaneously raising important questions about food safety, regulation, and public health. The dichotomy between street food's economic accessibility and potential health risks has made it a subject of growing academic interest, particularly in rapidly urbanizing regions of Africa, Asia, and Latin America where street vending employs substantial portions of the workforce and feeds diverse populations across socioeconomic strata. Research has demonstrated that street foods fulfill crucial roles in urban food security, providing employment opportunities for marginalized populations while offering convenient, culturally appropriate meals to consumers, yet their operations often exist in regulatory gray zones that complicate efforts to ensure safety standards without displacing vulnerable vendors. This multifaceted phenomenon demands interdisciplinary approaches that balance public health imperatives with socioeconomic realities, making street vended foods a compelling lens through which to examine broader issues of urban development, food governance, and the informal economy (Steyn *et al.,* 2014).

Foodborne pathogens represent one of the most significant public health challenges globally, causing an estimated 600 million illnesses and 420,000 deaths annually according to the World Health Organization. These microscopic adversaries including bacteria like *Salmonella, Escherichia coli O157,H7, and Listeria monocytogenes*, viruses such as *norovirus* and *hepatitis*

A, and *parasites* like *Toxoplasma gondii* have evolved sophisticated mechanisms to contaminate our food supply at multiple points from farm to fork. The emergence of antimicrobial-resistant strains, coupled with globalization of food trade and changing dietary patterns, has intensified surveillance efforts and sparked innovations in detection and prevention strategies. Research has highlighted the complex interplay between climate change and foodborne disease incidence, with temperature fluctuations affecting pathogen survival and proliferation rates in agricultural environments (Scallan *et al.,* 2011, Newell *et al.,* 2010).

Foodborne pathogens are ubiquitously found throughout the food production chain, from farm to fork, with their presence documented across multiple environmental and biological reservoirs. These microorganisms commonly inhabit soil, water sources, animal intestinal tracts, and raw agricultural products, with contamination occurring during primary production, processing, distribution, and food preparation stages (Newell *et al.,*2010). Pathogens such as *Salmonella, Escherichia coli O157,H7, Campylobacter*, and *Listeria monocytogenes* are frequently isolated from poultry, beef, pork, unpasteurized dairy products, fresh produce, and ready-to-eat foods (Scallan *et al.,* 2011). Cross-contamination through food contact surfaces, equipment, food handlers' hands, and inadequate sanitation practices significantly contributes to pathogen dissemination in retail and food service environments (Todd *et al.,* 2007). Additionally, environmental persistence in biofilms, refrigeration units, and processing plant drains creates ongoing contamination risks while emerging concerns include pathogen presence in irrigation water, organic fertilizers, and wildlife interfaces with agricultural systems. (Carpentier and Cerf, 2011.)

1.1 Aim and Objectives

The aim of this study was to determine the effect of food-borne pathogens isolated from street-vended foods on hematological parameters and oxidative stress markers of wistar rats

The specific objectives were to,

1. identify food-borne bacteria isolated from street vended foods using molecular techniques
2. determine the phenotypic virulence characteristics (biofilm formation, heamolysin and gelatinase production) of food borne bacteria isolates
3. enumerate, isolate and identify bacteria from rat feed samples
4. determine the weight gain/loss of wistar rats before and after infection with selected food-borne pathogens
5. determine the effect of food borne pathogens on oxidative stress markers (SOD, CAT, MDA and GPx levels) of wistar rats
6. determine the effect of food borne pathogen on hematological parameters of wistar rats

CHAPTER TWO

LITERATURE REVIEW

2.1 Street vended foods

Street-vended foods are ready-to-eat foods and beverages prepared and sold by vendors and hawkers, especially in streets and other public places, often outside formal food establishments like restaurants or cafes. They are typically consumed on-the-spot or taken away, and are popular due to their affordability, accessibility, and cultural relevance. “Foods and beverages prepared and sold by vendors in streets and public places, providing inexpensive, accessible, and often traditional meals to consumers.” (Oyet *et al.,* 2021.)

“Ready-to-eat foods and beverages prepared and/or sold by vendors especially on streets and other public places. “Street-vended foods are an integral part of urban food supply systems, meeting nutritional and social needs of urban populations, but often associated with public health risks due to contamination.” (Ajayi and Oluwoye 2015).

Street-vended foods ae ready-to-eat foods/drinks sold in public places outside formal food businesses. They are cheap, accessible, and culturally rooted, especially important for urban populations in Nigeria. However, they are often linked with contamination risks due to poor hygiene, unsafe handling, and inadequate regulation. Street foods are widely patronized in Nigeria but are commonly implicated in foodborne illnesses due to poor handling, preparation under unhygienic conditions, and lack of regulatory enforcement (Mchi *et al.,* 2023.)

Street-vended foods represent a complex, multifaceted phenomenon that encompasses ready-to-eat foods and beverages prepared, processed, and sold by vendors in streets, markets, and other public spaces across urban and peri-urban areas worldwide. The World Health Organization

(WHO, 1996) provides the foundational definition of street foods as "ready-to-eat foods and beverages prepared and/or sold by vendors or hawkers especially in the streets and other similar places for immediate consumption or consumption at a later time without further processing or preparation." This definition has been expanded and refined by numerous scholars to encompass the broader socio-economic, cultural, and nutritional dimensions of street food systems. The Food and Agriculture Organization (FAO, 2009) extends this conceptualization by emphasizing that street foods constitute an integral component of urban food systems, serving multiple functions including employment generation, food security enhancement, cultural preservation, and economic development in both developed and developing countries.

The characteristics of street-vended foods are inherently diverse and context-specific, varying significantly across geographical regions, cultural settings, and economic environments. However, several universal characteristics define this sector globally. (Steyn *et al.,* 2014) identify accessibility as a primary characteristic, noting that street foods are typically available in high-traffic areas where consumers congregate, including transportation hubs, commercial districts, educational institutions, and residential neighborhoods. Affordability represents another fundamental characteristic, with street foods generally priced significantly lower than restaurant meals, making them accessible to low income populations (Alimi, 2016). The convenience factor is equally important, as these foods require no further preparation and can be consumed immediately, catering to the fast-paced lifestyle of urban populations (Rane, 2011).

Cultural authenticity emerges as a defining characteristic, particularly in developing countries where street foods often represent traditional and indigenous food preparations passed down through generations. Omemu (2011) emphasizes that Nigerian street foods, for example, maintain strong cultural connections while adapting to urban environments and changing

consumer preferences. The informality of street food operations constitutes another key characteristic, with most vendors operating outside formal regulatory frameworks, often lacking proper licenses, standardized food safety protocols, and permanent infrastructure (Mensah *et al.,* 2012).

From a preparation and processing perspective, street-vended foods exhibit remarkable diversity in cooking methods, ingredients, and presentation styles. Traditional cooking techniques including frying, boiling, roasting, steaming, and grilling are commonly employed, often using locally available ingredients and traditional recipes (Okafor *et al.,* 2013). The use of minimal infrastructure is characteristic, with vendors typically operating with basic equipment such as portable stoves, simple cooking utensils, and makeshift serving areas. This operational simplicity allows for low barrier entry into the sector but also presents challenges regarding food safety and quality control.

The temporal and spatial characteristics of street food vending are equally significant. Many vendors operate during specific time periods, often aligning with meal times, work schedules, or seasonal patterns. The mobility of many street food operations allows vendors to follow consumer demand, moving between different locations throughout the day or week. Some vendors maintain fixed locations, establishing semi-permanent stalls or kiosks, while others remain highly mobile, using carts, bicycles, or carrying their wares on foot.

Street-vended foods, as comprehensively defined by the World Health Organization (WHO, 1996), represent ready-to-eat foods and beverages prepared and sold by vendors in public spaces for immediate consumption, constituting a critical component of global urban food systems that serves multiple socio-economic functions including employment generation, food security

enhancement, and cultural preservation (FAO, 2009). These foods are characterized by their high accessibility, typically available in high-traffic areas such as transportation hubs, commercial districts, and educational institutions where consumers congregate (Steyn *et al.,* 2014), exceptional affordability compared to formal restaurant meals, making them particularly important for low-income urban populations (Alimi, 2016), and remarkable convenience requiring no further preparation for immediate consumption, thus catering to fast-paced urban lifestyles (Rane, 2011). Street-vended foods maintain strong cultural authenticity by preserving traditional and indigenous food preparations while adapting to urban environments and changing consumer preferences (Omemu, 2011), operate predominantly within informal economic frameworks outside standardized regulatory systems (Mensah *et al.,* 2012), utilize diverse traditional cooking methods including frying, boiling, roasting, and steaming with locally available ingredients and minimal infrastructure consisting of portable equipment and makeshift serving areas (Okafor *et al.,* 2013), exhibit temporal and spatial flexibility through mobile operations that follow consumer demand patterns throughout different locations and time periods (Crush and Frayne, 2011), contribute significantly to urban nutrition by providing essential calories, proteins, and micronutrients despite quality and safety concerns (Abrahale *et al.,* 2019), support extensive value chains connecting smallholder farmers, ingredient suppliers, equipment manufacturers, and consumers within urban food networks (Kolawole *et al.,* 2013), face persistent challenges including inadequate infrastructure, limited access

to clean water and sanitation, regulatory harassment, and food safety concerns that impact both vendors and consumers (Ackah *et al.,* 2011), while simultaneously representing opportunities for entrepreneurship, skill development, and economic empowerment particularly for women and marginalized populations in developing countries (Adeleke *et al.,* 2020), thereby establishing

street-vended foods as a complex, multifaceted phenomenon that intersects food security, economic development, cultural preservation, and urban planning across diverse global contexts.

2.1 Historical and Cultural Context of Street-Vended Foods in Nigeria

2.1.1 Pre-Colonial Foundations and Indigenous Food Systems (Before 1861)

The historical trajectory of street-vended foods in Nigeria traces back to sophisticated pre-colonial trading systems and indigenous food cultures that established the foundational practices still evident in contemporary street food operations. Archaeological evidence suggests that organized food vending activities existed in major trading centers like Benin City, Kano, and Ile-Ife as early as the 11th century, where specialized food preparation and sale catered to merchants, travelers, and urban populations (Falola and Heaton, 2008). The pre-colonial period established fundamental characteristics of Nigerian street food culture including the predominance of women vendors, community-based food preparation methods, and the integration of street food sales with broader economic and social networks.

Traditional market systems across different ethnic regions developed distinct food vending practices that reflected local agricultural production, cultural preferences, and social organization. In Yorubaland, the "oja" (market) system incorporated specialized food sections where women prepared and sold foods like akara (bean fritters), moin-moin (steamed bean pudding), and various yam preparations to traders and community members (Bascom, 1969). The Hausa trading networks across the northern regions established rest stops and trading posts where foods like fura da nono (millet balls with fermented milk), kilishi (dried meat), and various grain-based preparations were sold to long-distance traders traversing trans-Saharan routes (Lovejoy, 1986).

Igbo communities developed "afor" and "eke" market day systems that rotated across different villages, creating opportunities for specialized food vendors to move between locations following predictable demand patterns (Ottenberg, 1959). These rotating market systems established mobility patterns and multi-location vending strategies that remain characteristic of contemporary Nigerian street food operations. The integration of food vending with traditional festivals, ceremonies, and social gatherings established cultural associations between specific foods and communal events that continue to influence consumer preferences and seasonal demand patterns.

Traditional preservation and processing techniques developed during the pre-colonial period laid technological foundations for street food preparation methods still widely used today. Smoking, drying, fermentation, and oil preservation techniques enabled vendors to extend shelf life and maintain food safety without refrigeration, while traditional cooking methods using clay pots, open fires, and steaming techniques shaped flavor profiles and preparation practices (Nwokolo, 1987). The development of specialized cooking equipment, serving vessels, and portable preparation tools reflected sophisticated understanding of mobile food preparation requirements adapted to outdoor and temporary vending environments.

2.1.2 Colonial Period Transformations (1861-1960)

The colonial period introduced profound transformations to Nigerian food systems and street vending practices through urbanization, new ingredients, changed labor patterns, and modified regulatory environments. British colonial administration concentrated populations in administrative centers like Lagos, Kano, Ibadan, and Port Harcourt, creating unprecedented urban food demand that stimulated growth in street food vending as rural-urban migrants sought

convenient, affordable meal options (Hopkins, 1973). Colonial wage labor systems altered traditional household food production and consumption patterns, increasing reliance on purchased meals and creating regular income flows that supported street food purchases.

The introduction of new crops and ingredients during the colonial period significantly expanded the repertoire of street-vended foods. Cassava, introduced from South America via Portuguese traders, became a fundamental ingredient in numerous street foods including garri (processed cassava flakes), akpu, and various cassava-based snacks (Jones, 1959). Maize introduction led to the development of roasted corn, corn-based porridges, and various corn snacks that became staples of Nigerian street food culture. Rice importation and local cultivation expansion created new categories of rice-based street foods including various rice and stew combinations that adapted traditional preparation methods to new ingredients.

Colonial transportation infrastructure development, including railway lines connecting major cities and ports, created new vending opportunities at transportation hubs while facilitating ingredient movement between regions (Ayodeji, 2001). Railway stations, motor parks, and port areas became major street food vending locations where vendors served travelers, workers, and passengers. The standardization of currency and expansion of monetized exchange systems replaced some barter-based food transactions with cash sales, enabling more systematic pricing and business development.

Colonial education systems and Christian missionary activities introduced new dietary concepts, hygiene practices, and food safety awareness that influenced street food preparation methods and consumer expectations. However, these influences often conflicted with traditional practices,

creating ongoing tensions between traditional food preparation methods and colonial health standards that continue to influence regulatory approaches to street food vending (Ranger, 1975).

2.1.3 Post-Independence Development and Urbanization (1960-1999)

Nigeria's independence in 1960 initiated rapid urbanization and economic development that fundamentally transformed street food vending from traditional market activities into major urban livelihood strategies. The oil boom of the 1970s accelerated urban migration and increased disposable income, expanding demand for street foods among growing urban working populations (Watts, 1987). Major cities like Lagos experienced explosive population growth, with rural-urban migrants bringing diverse regional food traditions that enriched urban street food varieties while creating new fusion foods that combined different ethnic culinary traditions.

Post-independence economic policies, including import substitution industrialization and later structural adjustment programs, significantly impacted street food systems. The 1980s economic crisis and subsequent structural adjustment programs implemented under military governments led to massive formal sector job losses, forcing many Nigerians into informal sector activities including street food vending (Lewis, 1996). Currency devaluations made imported food ingredients expensive while reducing purchasing power, leading to increased reliance on locally produced ingredients and making street foods more attractive due to their affordability compared to restaurant meals.

The expansion of educational institutions, particularly universities and polytechnics, created concentrated populations of students with limited income but regular food needs, establishing educational institutions as major street food markets. Campus and near-campus vending became specialized sectors with foods adapted to student preferences and budgets, including various

quick snacks, energy-dense meals, and affordable protein sources (Oguntona and Akinyele, 1995). Student food preferences influenced broader urban food trends, with campus-popular foods often spreading to general urban markets.

Industrial development during this period created factory worker populations requiring convenient, affordable meal options during work breaks, leading to the establishment of street food clusters around industrial areas.

Foods were adapted to industrial worker schedules and preferences, with emphasis on portable, energy-dense options that could be consumed quickly during limited break times (Andrae and Beckman, 1985). The growth of informal sector activities beyond food vending created symbiotic relationships between different informal businesses, with street food vendors serving other informal workers in concentrated informal business districts.

2.1.4 Contemporary Cultural Evolution and Globalization (2000-Present)

The contemporary period has witnessed unprecedented transformation of Nigerian street food culture through globalization, technology adoption, changing consumer preferences, and evolving urban dynamics. Digital technology integration has revolutionized street food operations through mobile payment systems, social media marketing, GPS-based location sharing, and online ordering platforms that expand market reach beyond traditional foot traffic (Adesoji, 2017). Social media platforms enable vendors to build brand recognition, advertise daily offerings, and engage with customers, while mobile payment systems reduce transaction costs and improve financial security.

Globalization has introduced new ingredients, cooking techniques, and business models that blend with traditional practices to create innovative street food offerings. International fast food

concepts have influenced presentation styles, packaging methods, and service approaches, while maintaining Nigerian flavor profiles and ingredients (Akinyemi and Adebayo, 2013). The emergence of "Afro-fusion" street foods combines traditional Nigerian preparations with international influences, creating new food categories that appeal to cosmopolitan urban consumers while maintaining cultural authenticity.

Diaspora connections have strengthened cultural dimensions of Nigerian street foods through international exposure and reverse cultural flows. Nigerians living abroad have created international markets for Nigerian street foods, while returning diaspora populations bring new expectations and international perspectives that influence local food evolution (Adepoju, 2004). International festivals and cultural events featuring Nigerian foods have increased global awareness and appreciation, contributing to cultural pride and preservation efforts.

Contemporary urbanization patterns, including the growth of mega-cities and urban sprawl, have created new spatial configurations for street food vending. Shopping mall integration, organized food courts, and designated street food zones represent formalization trends that maintain cultural authenticity while meeting modern health and safety standards (Mabogunje, 2002). The emergence of street food festivals, competitions, and cultural events demonstrates growing recognition of street foods as important cultural heritage requiring preservation and celebration.

2.2 Aspects of street vended foods

2.2.1 Nutritional and Health Dimensions

The nutritional profile of street-vended foods presents a complex landscape that varies significantly across regions, preparation methods, and ingredient availability. Comprehensive nutritional analyses reveal that street foods can provide substantial portions of daily caloric intake, often contributing 20-40% of total daily energy consumption among regular consumers (Steyn *et al.,* 2014). Protein content varies considerably, with meat-based street foods like Nigerian suya providing high-quality complete proteins containing essential amino acids, while plant-based options like roasted corn or plantain offer incomplete proteins that require complementary foods for optimal nutrition (Nwanekezi *et al.,* 2010).

Micronutrient profiles of street foods reflect both opportunities and challenges for public health nutrition. Traditional preparation methods often preserve important vitamins and minerals, particularly when fresh, locally sourced ingredients are used (Oyebode *et al.,* 2016). However, processing techniques involving high temperatures, extended cooking times, or oil reuse can reduce vitamin content, particularly heat-sensitive vitamins like vitamin C and certain B vitamins (Proietti *et al.,* 2013). Iron content tends to remain stable or even increase in foods cooked in iron pots, contributing to iron intake in populations at risk for iron deficiency anemia (Geerligs *et al.,* 2003).

The glycemic impact of street foods varies substantially based on ingredients and preparation methods. Carbohydrate-rich foods like fried plantain or rice-based dishes can cause rapid blood glucose elevation, while protein and fiber-rich options provide more sustained energy release (Brand-Miller *et al.,* 2003). Sodium content often exceeds recommended levels due to salt use

for preservation and flavor enhancement, contributing to concerns about hypertension and cardiovascular disease in urban populations (Campbell *et al.,* 2012).

2.2.2 Economic Impact and Market Dynamics

The economic significance of street-vended foods extends far beyond individual vendor incomes to encompass broader macroeconomic impacts including GDP contribution, employment generation, and poverty reduction. Conservative estimates suggest that street food sectors contribute 1-3% of GDP in developing countries, though actual contributions may be higher due to underreporting and informal nature of operations (ILO, 2018). Employment generation includes both direct employment for vendors and indirect employment throughout supply chains, with multiplier effects estimated at 2-3 additional jobs for every direct street food vendor position (Bhowmik, 2012).

Market dynamics within street food sectors demonstrate sophisticated pricing strategies, location optimization, and demand forecasting despite operating outside formal business frameworks. Vendors employ complex pricing mechanisms that account for ingredient costs, competition, consumer purchasing power, and seasonal variations (Tinker, 1997). Location selection involves careful analysis of foot traffic patterns, competition density, regulatory risks, and infrastructure availability, with premium locations commanding higher prices but also generating higher revenues (Cross, 2000).

Supply chain relationships reveal extensive networks connecting rural producers, urban wholesalers, processing facilities, and retail vendors through both formal and informal channels. These relationships often involve credit arrangements, quality agreements, and mutual support systems that facilitate market access for smallholder producers while ensuring reliable supply for

vendors (Reardon and Gulati, 2008). Forward linkages connect street food vendors to consumers through direct sales, repeat customer relationships, and increasingly through digital platforms and delivery services.

2.2.3 Regional Cultural Variations and Ethnic Diversity

Nigeria's cultural diversity is richly reflected in regional street food variations that maintain distinct ethnic identities while creating opportunities for cross-cultural culinary exchange in urban environments. Northern Nigerian street food culture, dominated by Hausa-Fulani traditions, emphasizes grilled meats, grain-based preparations, and dairy products adapted to semi-arid climatic conditions and pastoralist lifestyles (Blench, 2013). Suya preparation represents sophisticated meat preservation and flavoring techniques using traditional spice blends (yaji) that combine local and trans-Saharan trade-derived ingredients.

Southwestern Nigerian street food culture reflects Yoruba agricultural abundance and complex urban trading traditions. Foods like akara, plantain preparations, and various yam dishes demonstrate sophisticated fermentation, frying, and flavor combination techniques that maximize nutritional value and shelf life (Oyewole, 1997).

The integration of palm oil, locust beans, and various indigenous spices creates distinctive flavor profiles that remain culturally significant while adapting to urban consumer preferences and time constraints.

Southeastern Nigerian street food traditions emphasize root crops, palm products, and protein sources reflecting Igbo agricultural practices and trading acumen. Foods like abacha (African salad), ukwa (breadfruit), and various cassava preparations demonstrate complex preparation techniques that transform basic ingredients into highly valued cultural foods (Nnanyelugo and

Ngoddy, 1985). The adaptation of traditional feast foods for street vending maintains cultural connections while creating commercial opportunities for urban Igbo populations.

Middle Belt and minority ethnic group contributions to Nigerian street food culture demonstrate remarkable diversity and creativity in adapting local ingredients and traditional preparations for urban markets. Foods from Plateau, Benue, and other Middle Belt states introduce unique ingredients and preparation methods that enrich national street food diversity while maintaining distinct cultural identities (Korieh, 2005). The interaction between different ethnic food traditions in urban areas creates fusion foods that represent contemporary Nigerian multicultural urban identity.

2.2.4 Gender, Age, and Social Dynamics

Street food vending in Nigeria demonstrates complex gender dynamics that reflect traditional role divisions while creating opportunities for female economic empowerment and social mobility. Women constitute approximately 70-80% of street food vendors across Nigerian cities, reflecting cultural associations between women and food preparation as well as limited formal sector employment opportunities (Tinker, 1997). Female dominance in street food vending creates important income generation opportunities while maintaining cultural appropriateness within traditional gender role expectations.

Intergenerational knowledge transfer represents crucial mechanisms for maintaining traditional food preparation techniques and cultural authenticity. Older women often train younger female relatives in specialized food preparation techniques, business skills, and customer relationship management, creating informal apprenticeship systems that preserve cultural knowledge while

developing human capital (Brown, 2000). These knowledge transfer systems maintain quality standards and cultural authenticity while enabling business expansion and succession planning.

Age-based specialization patterns demonstrate strategic market segmentation based on physical capabilities, capital requirements, and social networks. Younger women often specialize in labor-intensive foods requiring physical strength and mobility, while older women focus on specialized preparations requiring extensive experience and established customer relationships (Osibanjo and Abiodun, 2013). Middle-aged women often achieve optimal market positions through combinations of experience, physical capability, and established business networks.

Social capital formation through street food vending creates important community networks that provide mutual support, information sharing, and collective action capabilities. Vendor associations, traditional savings groups (esusu), and informal credit systems demonstrate sophisticated social organization that enables risk management and business development (Adekanye, 1988). These social networks extend beyond economic functions to provide social support, conflict resolution, and community development initiatives.

2.2.5 Contemporary Challenges and Cultural Preservation

Contemporary street food culture in Nigeria faces significant challenges from urbanization pressures, regulatory changes, health concerns, and changing consumer preferences that threaten traditional practices while creating opportunities for innovation and adaptation.

Urban development projects, road construction, and city beautification efforts often displace traditional street food vending locations, disrupting established customer relationships and forcing vendors to adapt to new environments (Jimu, 2004). The tension between urban planning

objectives and informal sector accommodation remains largely unresolved, creating ongoing insecurity for street food vendors.

Food safety regulations and health authority interventions reflect growing public health awareness while often conflicting with traditional preparation methods and operational practices. The challenge of maintaining cultural authenticity while meeting modern food safety standards requires innovative approaches that respect traditional knowledge while incorporating contemporary hygiene practices (WHO, 2006). Training programs, infrastructure improvements, and supportive regulatory frameworks represent potential solutions that balance cultural preservation with public health protection.

Changing consumer preferences, particularly among younger, educated populations, create both challenges and opportunities for traditional street food vendors. Increased health consciousness, dietary diversification, and international food exposure influence demand patterns while creating opportunities for menu innovation and presentation improvements (Popkin, 2001). The adaptation of traditional foods to meet contemporary health and convenience expectations demonstrates cultural resilience and creative adaptation capabilities.

Cultural preservation efforts including documentation projects, cooking competitions, and cultural festivals demonstrate growing recognition of street food cultural importance and the need for systematic preservation initiatives. Academic research, government cultural programs, and non-governmental organization initiatives increasingly focus on street food culture as important intangible cultural heritage requiring protection and promotion (UNESCO, 2003). These efforts create opportunities for cultural validation, tourism development, and economic empowerment while maintaining authentic cultural practices.

2.3 Types of Street-Vended Foods in Nigeria

Traditional Nigerian street snacks represent a diverse category of indigenous food preparations that maintain deep cultural connections while adapting to urban commercial environments and contemporary consumer preferences. These snacks are characterized by their portability, extended shelf life, distinctive flavor profiles, and cultural authenticity that connects urban consumers with traditional food heritage (Nnam, 2001). The classification of traditional street snacks reflects Nigeria's remarkable ethnic diversity, with different regions contributing unique preparations based on local ingredients, traditional cooking methods, and cultural preferences that have evolved over centuries of culinary development.

The cultural significance of traditional street snacks extends beyond simple nutrition provision to encompass identity preservation, social interaction, cultural transmission, and economic empowerment for predominantly female vendors who maintain traditional preparation knowledge. (Oguntona and Akinyele, 1995) emphasize that traditional street snacks serve as vehicles for preserving indigenous food knowledge, traditional processing techniques, and cultural food practices that might otherwise disappear in rapidly modernizing urban environments. The preparation and consumption of traditional snacks create opportunities for intergenerational knowledge transfer, cultural celebration, and community bonding that strengthen social cohesion in diverse urban settings.

Regional variations in traditional street snacks reflect Nigeria's complex ethnic geography, with distinct snack traditions emerging from Yoruba, Hausa-Fulani, Igbo, and numerous minority ethnic groups that contribute unique ingredients, preparation methods, and cultural associations. These regional variations create rich diversity within Nigerian street food culture while

demonstrating remarkable adaptation capabilities as regional specialties spread to different urban centers through migration, cultural exchange, and commercial opportunities (Oyewole, 1997). The fusion and adaptation of traditional snacks in multi-ethnic urban environments creates new varieties that blend traditional authenticity with contemporary preferences and cross-cultural influences.

2.3.1 Grain-Based Traditional Snacks

Grain-based traditional snacks constitute a fundamental category of Nigerian street foods, utilizing indigenous and introduced grains through sophisticated processing techniques that maximize nutritional value, shelf stability, and palatability. These snacks demonstrate remarkable diversity in grain utilization, processing methods, and flavor combinations that reflect both traditional food technology and adaptive innovation in response to urban market demands and consumer preferences.

Akara (Bean Fritters) represents one of Nigeria's most iconic traditional street snacks, prepared from black-eyed peas (Vigna unguiculata) through complex processing involving soaking, dehulling, grinding, seasoning, and deep-frying techniques that create distinctive texture, flavor, and nutritional profiles. Comprehensive analysis by (Adebowale *et al*., 2005) demonstrates that akara provides substantial protein content (18-22% dry weight), essential amino acids, B-vitamins, and minerals while maintaining cultural authenticity through traditional preparation methods passed down through generations. The preparation process involves soaking beans for 3-6 hours, manual or mechanical dehulling, grinding with traditional grinding stones or modern blenders, seasoning with onions, peppers, and salt, and deep-frying in palm oil or vegetable oil at controlled temperatures to achieve optimal texture and flavor development.

Regional variations in akara preparation demonstrate sophisticated adaptation to local preferences and ingredient availability, with different regions employing varying spice combinations, oil types, grinding techniques, and serving methods that create distinct local identities while maintaining core preparation principles. Lagos-style akara often incorporates more complex spice blends including ginger, garlic, and hot peppers, while northern Nigerian versions may include traditional Hausa spices and different oil preferences (Omemu and Faniran, 2011). The commercial success of akara as a street food reflects its optimal combination of nutritional value, cultural significance, preparation feasibility, and consumer acceptance across diverse demographic groups.

Kosai (Hausa Bean Cakes) represents the northern Nigerian equivalent of akara, demonstrating regional adaptation of bean-based snack preparation with distinct cultural characteristics and preparation methods that reflect Hausa-Fulani food traditions and preferences. Kosai preparation involves similar basic processes as akara but incorporates different spice combinations, cooking techniques, and serving methods that create distinct flavor profiles and cultural associations (Nkama and Gbenyi, 2001). The use of traditional Hausa spices including dried ginger, cloves, and regional pepper varieties creates unique taste characteristics that distinguish kosai from southern Nigerian akara preparations.

The nutritional profile of kosai demonstrates similar protein and micronutrient contributions as akara while reflecting regional ingredient variations and preparation modifications that influence nutrient retention and bioavailability. Studies by (Muhammad *et al.,* 2011) indicate that kosai provides comparable protein quality to akara while offering distinct mineral profiles reflecting regional soil conditions, processing variations, and traditional ingredient combinations. The cultural significance of kosai extends beyond nutrition to encompass Hausa identity, traditional

celebration foods, and social gathering practices that strengthen community connections and cultural continuity.

Masa (Rice Cakes) represents a sophisticated traditional snack utilizing rice flour through fermentation processes that create unique texture, flavor, and nutritional characteristics while demonstrating advanced traditional food technology and cultural food processing knowledge. Masa preparation involves rice soaking, grinding, fermentation for 24-72 hours, seasoning, and cooking in specialized traditional pans that create distinctive round shapes and texture profiles (Gaffa *et al.,* 2002). The fermentation process develops complex flavors, improves protein quality, increases B-vitamin content, and enhances digestibility while creating characteristic tangy taste that distinguishes masa from other rice-based preparations.

Regional variations in masa preparation reflect different rice varieties, fermentation techniques, seasoning combinations, and cooking methods that create diverse taste profiles and cultural associations across northern Nigerian communities. Traditional masa preparation often incorporates potash (kanwa) that influences pH, fermentation patterns, mineral content, and final product characteristics while reflecting indigenous food technology knowledge developed over centuries of culinary experimentation and refinement (Akinrele and Edwards, 1971). The commercial viability of masa as street food demonstrates successful adaptation of traditional fermentation technology to urban commercial environments while maintaining cultural authenticity and nutritional benefits.

2.3.2 Root Crop-Based Traditional Snacks

Root crop-based traditional snacks utilize Nigeria's abundant tuber production through diverse processing techniques that create portable, shelf-stable, and nutritionally valuable snacks while maintaining cultural connections to traditional agricultural systems and food preparation methods.

These snacks demonstrate sophisticated understanding of root crop processing, preservation techniques, and flavor development that maximize both nutritional value and commercial viability in urban street food markets.

Plantain Chips (Ipekere) represent widespread traditional snacks utilizing green plantains through slicing, seasoning, and deep-frying processes that create crispy, flavorful, and nutritionally dense snacks with excellent shelf stability and consumer appeal. Traditional plantain chip preparation involves selecting appropriate maturity levels, precision slicing techniques, optional seasoning applications, and controlled frying processes that optimize texture, flavor, and nutritional retention (Adeniji *et al.,* 2006). The transformation of fresh plantains into shelf-stable chips demonstrates sophisticated traditional food technology that enables value addition, seasonal preservation, and commercial distribution of perishable agricultural products.

Nutritional analysis reveals that plantain chips provide substantial carbohydrate content (65-75% dry weight), moderate protein levels, essential minerals including potassium, magnesium, and vitamin C, while offering convenient energy-dense nutrition for urban consumers with limited meal preparation time (Akubor, 2003). The glycemic index of plantain chips varies based on maturity levels, processing methods, and preparation techniques, with green plantain chips generally offering more sustained energy release compared to ripe plantain preparations.

Regional and seasonal variations in plantain chip preparation reflect local preferences, plantain variety availability, processing equipment differences, and cultural seasoning traditions that create diverse product characteristics while maintaining core preparation principlized cutting techniques that create distinct local identities and market differentiation (Ogazi, 1996). The seasonal availability of plantains influences pricing, quality characteristics, and vendor business strategies while creating opportunities for value-added processing that extends market seasons and improves vendor income stability.

Cassava-Based Snacks (Garri, Tapioca) demonstrate sophisticated processing of cassava roots into diverse snack forms that provide essential carbohydrates while showcasing traditional detoxification and preservation techniques developed to manage cassava's natural cyanogenic compounds. Garri preparation involves complex processing including peeling, grating, fermentation, dewatering, sieving, and roasting that removes toxic compounds while creating distinctive flavor, texture, and shelf-stable characteristics (Nweke *et al.,* 2002). The fermentation process typically lasts 3-5 days and develops organic acids that contribute to flavor development, preservation, and improved digestibility while reducing cyanogenic glycoside content to safe consumption levels.

The nutritional significance of cassava-based snacks provides essential carbohydrates, moderate protein, and various micronutrients while offering affordable energy sources for low-income urban populations. Garri typically contains 80-85% carbohydrates, 2-4% protein, essential minerals, and B-vitamins developed during fermentation processes (Okafor *et al.,* 2003). The high energy density and extended shelf life make cassava-based snacks particularly valuable for food security, emergency nutrition, and convenient energy provision in urban environments with limited refrigeration infrastructure.

Processing variations create diverse cassava-based snack products including white garri, yellow garri (with palm oil addition), tapioca pearls, cassava flour products, and flavored preparations that cater to different consumer preferences, regional tastes, and market segments.

Yellow garri preparation incorporates palm oil during roasting processes, adding vitamin A precursors, distinctive flavor, and cultural preferences while demonstrating adaptive innovation within traditional processing frameworks (Essers *et al.,* 1995). These product variations enable market differentiation, consumer choice, and business specialization opportunities for street food vendors.

2.3.3 Protein-Rich Traditional Snacks

Protein-rich traditional snacks address urban nutrition needs through diverse animal and plant protein sources processed using traditional techniques that maximize protein quality, shelf stability, and palatability while maintaining cultural authenticity and commercial viability. These snacks demonstrate sophisticated protein processing knowledge, preservation techniques, and flavor development that create nutritionally dense options for urban consumers with limited access to fresh protein sources.

Suya (Spiced Grilled Meat) represents Nigeria's most internationally recognized traditional street snack, utilizing various meat types through complex spice blending, marination, and grilling techniques that create distinctive flavors, textures, and cultural associations. Traditional suya preparation involves meat selection (typically beef, although chicken, ram, and other meats are used), precise cutting into strips, application of traditional spice blend (yaji), marination periods, and grilling over open wood fires that impart characteristic smoky flavors (Igene *et al.,* 1990). The yaji spice blend typically includes ground groundnuts, ginger, cloves, cinnamon, red pepper,

garlic, onion powder, and other traditional spices that create complex flavor profiles while providing antimicrobial properties that enhance food safety.

Nutritional analysis demonstrates that suya provides high-quality complete protein (25-35% by weight), essential amino acids, B-vitamins, iron, zinc, and other minerals while offering convenient protein sources for urban consumers. The grilling process reduces moisture content, concentrates nutrients, and creates appealing textures while traditional spice applications provide antioxidants and antimicrobial compounds that contribute to food safety and nutritional value (Apata *et al.,* 2013). However, concerns regarding high sodium content, potential carcinogenic compound formation during grilling, and food safety practices require attention in commercial preparation and consumption recommendations.

Regional variations in suya preparation reflect different meat preferences, spice blend compositions, grilling techniques, and serving methods that create distinct local identities while maintaining core preparation principles. Northern Nigerian suya traditions often emphasize beef preparation with specific Hausa spice combinations, while southern adaptations may incorporate different meat types, modified spice blends, or alternative cooking methods (Nkama and Gbenyi, 2001). The adaptation of suya preparation to urban commercial environments demonstrates successful scaling of traditional food technology while maintaining cultural authenticity and consumer appeal.

Kilishi (Dried Spiced Meat) represents sophisticated traditional meat preservation technology that creates shelf-stable, protein-dense snacks through controlled drying, spicing, and preservation processes adapted to hot, arid climatic conditions of northern Nigeria. Kilishi preparation involves meat selection, precise slicing into thin sheets, spice application, controlled

drying under specific environmental conditions, and optional smoking processes that create distinctive texture, flavor, and preservation characteristics (Igene *et al.,* 1990). The traditional preparation process can extend meat preservation for several months without refrigeration while maintaining nutritional value and creating concentrated protein sources suitable for travel, storage, and commercial distribution.

The spice applications in kilishi serve multiple functions including flavor development, antimicrobial preservation, antioxidant protection, and cultural identity while demonstrating sophisticated understanding of traditional food preservation principles. Common spice ingredients include ginger, cloves, black pepper, garlic, and traditional northern Nigerian spices that create complex flavor profiles while providing natural preservation compounds that extend shelf life and enhance food safety (Alonge, 1991). The controlled drying process reduces moisture content to levels that prevent bacterial growth while concentrating nutrients and creating distinctive chewy texture characteristics.

Commercial adaptation of kilishi production demonstrates successful scaling of traditional preservation technology to urban market demands while maintaining cultural authenticity and nutritional benefits. Modern kilishi production may incorporate improved hygiene practices, standardized processing conditions, and quality control measures while preserving traditional spice formulations and preparation principles (Mohammed *et al.,* 2007). The high protein density, extended shelf life, and cultural significance make kilishi valuable for both local consumption and potential export markets interested in traditional African preserved foods.

2.3.4 Fermented and Processed Traditional Snacks

Fermented traditional snacks demonstrate sophisticated biotechnology knowledge developed through centuries of indigenous food processing experience, creating unique flavors, textures, nutritional profiles, and preservation characteristics while showcasing traditional fermentation expertise and microbial management understanding. These snacks utilize controlled fermentation processes that enhance nutritional value, improve digestibility, develop complex flavors, and create shelf-stable products suitable for commercial street food distribution.

Ogi/Pap-Based Snacks utilize fermented cereal-based batters prepared from corn, millet, or sorghum through controlled fermentation processes that create smooth, digestible, and nutritionally enhanced products suitable for various consumer groups including infants, elderly individuals, and adults seeking easily digestible nutrition. Traditional ogi preparation involves grain soaking, wet grinding, sieving, fermentation for 48-72 hours, and optional cooking processes that develop characteristic tangy flavors while improving protein quality and mineral bioavailability (Banigo and Muller, 1972). The fermentation process develops beneficial lactic acid bacteria that contribute to gut health, create natural preservation acids, and synthesize B-vitamins that enhance nutritional value.

Nutritional benefits of fermented ogi include improved protein digestibility, increased B-vitamin content, enhanced mineral bioavailability, reduced antinutrient levels, and prebiotic effects that support digestive health. Research by (Adeyemi and Beckley, 1986) demonstrates that fermentation processes increase protein efficiency ratio, reduce phytic acid content, and create amino acid profiles more suitable for human nutrition requirements. The smooth texture and mild

flavor make ogi-based preparations particularly suitable for young children, elderly consumers, and individuals with digestive sensitivities.

Commercial adaptations include instant ogi powders, flavored variants, fortified versions, and ready-to-consume preparations that maintain fermentation benefits while improving convenience, shelf stability, and market accessibility. Value-added ogi products may incorporate additional nutrients, natural flavoring, improved packaging, and standardized processing that creates premium market opportunities while preserving traditional fermentation principles (Sanni *et al.,* 1999).

Locust Bean-Based Products (Dadawa/Iru) represent sophisticated protein fermentation technology that transforms locust bean seeds into highly nutritious, flavorful condiments and snack components through controlled alkaline fermentation processes. Traditional dadawa preparation involves locust bean cooking, dehulling, fermentation with specific bacterial cultures (primarily Bacillus subtilis), and drying processes that create distinctive flavor composure

2.3.5 Rice-Based Cooked Meals

Rice-based cooked meals constitute a dominant category of Nigerian street foods, reflecting rice's status as a staple carbohydrate source and the versatility of rice preparations in accommodating diverse protein sources, vegetables, and flavoring systems that create distinctive meal combinations suitable for different consumer preferences and price points.

Jollof Rice represents Nigeria's most internationally recognized cooked meal, demonstrating sophisticated rice cooking techniques that combine long-grain rice with tomato-based sauce, traditional spices, vegetables, and optional protein sources to create flavorful, nutritionally balanced meals with distinctive orange coloration and complex taste profiles.

Traditional jollof rice preparation involves rice parboiling, sauce development using tomatoes, onions, peppers, and traditional spices, rice integration with sauce, controlled cooking processes that achieve optimal texture and flavor absorption, and final additions of vegetables and proteins (Sanni *et al.,* 2013). The cooking process typically requires 45-90 minutes and involves multiple stages including ingredient preparation, sauce cooking, rice integration, and flavor development that create characteristic taste, texture, and appearance.

Nutritional analysis demonstrates that jollof rice provides substantial carbohydrate content (55-65% of calories), moderate protein levels depending on added ingredients, essential B-vitamins from enriched rice, lycopene from tomatoes, and various micronutrients from vegetable additions. When prepared with meat, fish, or poultry, jollof rice becomes a complete meal providing all essential amino acids, while vegetarian versions require protein complementation through beans, nuts, or dairy additions (Adebowale *et al.,* 2012). The glycemic impact varies based on rice type, cooking methods, and accompanying ingredients, with brown rice versions offering improved glycemic control and additional fiber content.

Regional variations in jollof rice preparation create distinctive local identities while maintaining core preparation principles, with different regions employing varying spice combinations, protein preferences, vegetable additions, and cooking techniques. Lagos-style jollof often incorporates seafood, particularly prawns and fish, reflecting coastal location and fishing traditions, while northern Nigerian versions may emphasize meat preparations and different spice profiles reflecting Hausa culinary traditions (Nkama and Gbenyi, 2001). The ongoing "jollof wars" between Nigeria and Ghana regarding preparation authenticity demonstrates the cultural significance and national identity associated with this dish.

Fried Rice represents adaptation of Asian cooking techniques to Nigerian ingredients and taste preferences, creating fusion meals that combine stir-frying methods with local vegetables, proteins, and seasonings to produce colorful, nutritious, and appealing cooked meals. Nigerian fried rice preparation typically involves pre-cooked rice, mixed vegetables (carrots, green beans, peas, sweet corn), protein sources (chicken, beef, shrimp), eggs, and Nigerian seasonings including curry powder, thyme, and local spices (Ihekoronye and Ngoddy, 1985). The stir-frying process requires high heat, quick cooking, and sequential ingredient addition that preserves vegetable texture, color, and nutritional value while creating complex flavor combinations.

The nutritional profile of fried rice demonstrates balanced macronutrient distribution with moderate carbohydrate content, substantial protein from multiple sources, essential fatty acids from cooking oils, and diverse micronutrients from vegetable combinations. The inclusion of multiple vegetables provides vitamin A from carrots, vitamin C from peppers, folate from leafy greens, and various minerals that enhance overall nutritional value (Oguntona *et al.,* 1998). The cooking method preserves heat-sensitive vitamins better than prolonged boiling while creating appealing textures and flavors that attract diverse consumer groups.

Rice and Stew Combinations represent flexible meal systems that allow vendors to offer diverse protein and sauce options with rice as the carbohydrate base, creating customizable meals that accommodate different preferences, dietary restrictions, and budget constraints. Common stew varieties include tomato-based meat stew, fish stew, vegetable stew, okra stew, and various traditional sauce preparations that provide different nutritional profiles and taste experiences (Oyewole, 1997). This system enables efficient commercial operation through batch cooking of

rice and multiple stew options, allowing customers to select preferred combinations while maximizing vendor efficiency and ingredient utilization.

The nutritional advantages of rice and stew combinations include protein variety from different stew types, vegetable diversity from various sauce preparations, balanced macronutrient profiles, and cultural authenticity through traditional sauce recipes. Vendors can accommodate various dietary needs including vegetarian options, low-fat preparations, and different protein preferences while maintaining operational efficiency and cost control (Omemu, 2011). The flexibility of this system makes it particularly popular among street food vendors serving diverse urban populations with varying preferences and economic circumstances.

2.3.6 Bean-Based Cooked Meals

Bean-based cooked meals provide essential plant proteins, dietary fiber, and micronutrients while offering affordable, culturally authentic dining options that demonstrate sophisticated legume cooking techniques and traditional flavor development methods adapted to commercial street food preparation.

Moin-Moin (Steamed Bean Pudding) represents sophisticated bean processing and cooking technology that transforms black-eyed peas into smooth, protein-rich steamed puddings with complex flavors and appealing presentations. Traditional moin-moin preparation involves bean soaking, dehulling, grinding with peppers and onions, seasoning addition, optional ingredient incorporation (eggs, fish, meat), wrapping in banana leaves or aluminum foil, and steaming for 45-90 minutes until firm texture develops (Adeyemi and Idowu, 1990). The grinding process creates smooth batter consistency while maintaining protein structure, and steaming cooking method preserves nutritional value while creating distinctive texture and flavor characteristics.

Nutritional analysis reveals that moin-moin provides high-quality plant protein (15-20% by weight), essential amino acids, dietary fiber, B-vitamins particularly folate, iron, potassium, and other minerals essential for human nutrition. The protein quality approaches that of animal proteins when properly prepared, making moin-moin valuable for vegetarian diets and protein-limited food systems (Nwokolo, 1987). The steaming cooking method minimizes nutrient losses while creating digestible protein forms suitable for various age groups including children and elderly consumers.

Cultural significance extends beyond nutrition to encompass traditional celebrations, religious observances, and social gatherings where moin-moin serves as special occasion food representing hospitality, cultural identity, and culinary expertise. The preparation process often involves community participation, knowledge sharing, and cultural transmission that strengthens social bonds while preserving traditional food technology (Brown, 2000). Street vendors who specialize in moin-moin often maintain family recipes and preparation techniques passed down through generations while adapting to commercial requirements and modern consumer expectations.

2.3.7 Yam-Based Cooked Meals

Yam-based cooked meals utilize Nigeria's abundant yam production through diverse cooking methods that create nutritious, filling, and culturally significant meals while demonstrating traditional tuber processing knowledge and adaptation to urban commercial food preparation.

Pounded Yam with Soup represents traditional meal systems that combine labor-intensive yam processing with diverse soup preparations to create complete meals with cultural significance, nutritional balance, and distinctive eating experiences. Pounded yam preparation involves yam

boiling until tender, pounding with traditional mortars and pestles until smooth, elastic consistency develops, and serving with various traditional soups including egusi, okra, vegetable, or meat soups (Onwuka, 2005). The pounding process requires significant physical effort and skill to achieve proper texture, consistency, and appearance that meets cultural standards and consumer expectations.

Traditional soup preparations demonstrate remarkable diversity and sophistication, with egusi soup utilizing ground melon seeds, leafy vegetables, meat or fish, and traditional seasonings to create protein-rich, flavorful accompaniments that complement pounded yam's bland taste and starchy texture. Nutritional analysis reveals that pounded yam provides substantial carbohydrate content (80-85% of calories), moderate protein, potassium, vitamin C, and dietary fiber, while accompanying soups contribute protein, vitamins, minerals, and essential fatty acids that create balanced nutritional profiles (Tortoe, 2010). The combination provides complete meals suitable for heavy physical work and active lifestyles common in urban environments.

Cultural significance encompasses traditional dining customs, social eating practices, and ceremonial meals that strengthen community bonds and cultural identity while creating economic opportunities for vendors specializing in traditional meal preparation. The labor-intensive preparation process often requires specialized skills, equipment, and time investments that create barriers to entry while enabling premium pricing for vendors who master traditional techniques (Afoakwa and Sefa-Dedeh, 2001).

Street vendors often adapt traditional preparation methods using mechanical equipment while maintaining cultural authenticity and consumer acceptance.

Boiled Yam with Various Sauces provides simplified preparation methods that maintain yam's nutritional benefits while reducing preparation time and complexity for commercial street food operations. Boiled yam preparation involves yam peeling, cutting, boiling until tender, and serving with diverse sauce options including tomato stew, palm oil sauce, pepper sauce, or traditional soup preparations (Eka, 1985). The simplified preparation enables vendors to offer yam-based meals without specialized equipment or extensive preparation time while maintaining nutritional value and cultural acceptability.

Sauce variety creates meal customization opportunities that accommodate different preferences, dietary restrictions, and budget constraints while maximizing ingredient utilization and vendor efficiency. Common sauce options include tomato-based meat or fish stew, vegetable sauces, palm oil with pepper and onions, groundnut soup, and various traditional preparations that provide protein, vitamins, and flavor enhancement (Oke, 1965). The nutritional contribution varies significantly based on sauce selection, with protein-rich sauces creating complete meals while simpler preparations provide primarily carbohydrate nutrition requiring protein complementation.

Yam Porridge (Asaro) demonstrates one-pot cooking methods that combine yam with vegetables, proteins, and seasonings to create complete, nutritious, and flavorful meals using efficient cooking processes suitable for commercial street food preparation. Asaro preparation involves yam cutting, cooking with palm oil, onions, peppers, tomatoes, leafy vegetables, optional protein additions (fish, meat), and traditional seasonings until yam becomes partially mashed while maintaining texture variety (Ogazi, 1996). The cooking process typically requires 30-45 minutes and creates complex flavor combinations while preserving nutritional value from diverse ingredients.

Nutritional advantages include balanced macronutrient profiles from yam carbohydrates and added proteins, diverse micronutrients from vegetable additions, essential fatty acids from palm oil, and dietary fiber supporting digestive health and satiety. The one-pot preparation method preserves water-soluble vitamins while creating appealing flavors and textures that attract consumers seeking nutritious, convenient meals (Onwuka, 2005). The flexibility of ingredient additions enables vendors to accommodate various preferences, seasonal availability, and cost considerations while maintaining meal quality and cultural authenticity.

2.3.8 Meat and Fish-Based Cooked Meals

Meat and fish-based cooked meals provide high-quality animal proteins, essential amino acids, and important micronutrients while demonstrating sophisticated protein cooking techniques, preservation methods, and flavor development systems adapted to street food commercial requirements and food safety considerations.

Pepper Soup represents traditional medicinal cooking that combines meat or fish with traditional spices, herbs, and peppers to create flavorful, protein-rich meals with cultural significance and perceived health benefits. Traditional pepper soup preparation varies by region and protein source but typically involves protein cleaning, seasoning, cooking with traditional spice combinations including uda seeds, uziza leaves, utazi leaves, ginger, and hot peppers until tender, creating aromatic, spicy broths with distinctive flavors (Achi, 2005). The cooking process typically requires 1-2 hours and develops complex flavor profiles while preserving protein quality and nutritional value.

Nutritional analysis demonstrates high protein content (20-30% of calories), complete amino acid profiles, B-vitamins particularly B12 from animal proteins, iron, zinc, and various minerals essential for human nutrition.

2.4 Socio-Economic Importance of Street Food Vending

2.4.1 Employment Generation and Labor Market Dynamics

Street food vending represents one of the most significant sources of employment in developing economies, providing direct livelihood opportunities for millions of individuals while generating extensive indirect employment throughout associated supply chains and support systems. The International Labour Organization (ILO, 2018) estimates that street food vending directly employs approximately 2.5 billion people globally, with particularly high concentrations in sub-Saharan Africa, South Asia, and Latin America where formal sector employment opportunities remain limited. In Nigeria specifically, research by (Omemu and Aderoju, 2008) indicates that street food vending provides direct employment for approximately 2.8 million individuals, representing nearly 15% of total informal sector employment and constituting a critical livelihood strategy for urban and peri-urban populations.

The employment generation effects extend far beyond direct vending activities to encompass extensive backward and forward linkages that create multiplier effects throughout local and regional economies. Backward linkages include agricultural production, food processing, packaging, equipment manufacturing, transportation, and wholesale distribution, while forward linkages encompass waste management, equipment maintenance, financial services, and complementary retail activities (Bhowmik, 2005). Economic impact assessments suggest employment multiplier effects ranging from 2.5 to 4.2 additional jobs created for every direct

street food vendor position, indicating substantial indirect employment generation that supports broader economic development objectives (Roever, 2014).

Gender dimensions of street food employment reveal both opportunities and challenges for women's economic participation and empowerment. Cross-national studies demonstrate that women constitute 60-90% of street food vendors across different regions, with particularly high female participation rates in sub-Saharan Africa where women represent approximately 75-85% of street food vendors (Chen, 2012). This female dominance reflects cultural associations between women and food preparation, limited formal sector opportunities for women, and the compatibility of street food vending with domestic responsibilities and childcare obligations (Brown, 2006).

Age-based employment patterns demonstrate street food vending's role as both entry-level employment for young people and essential income security for older workers facing formal sector age discrimination. Youth employment in street food vending often serves as entrepreneurship training, skill development, and capital accumulation opportunities that enable progression to other economic activities or business expansion (Lyons and Snoxell, 2005). For older workers, particularly those lacking formal education or technical skills, street food vending provides essential income security and social protection in contexts with limited formal social security systems (Lloyd-Evans, 2008).

Skills development and human capital formation within street food sectors demonstrate sophisticated informal training systems that develop business management, customer service, food safety, financial management, and marketing capabilities. These skills are largely transferable to other economic activities and contribute to overall human capital development

within communities (King, 1996). Apprenticeship systems, peer learning networks, and family-based knowledge transfer create comprehensive skill development opportunities that operate outside formal education systems while providing practical, market-relevant capabilities.

2.4.2 Income Generation and Poverty Alleviation

Street food vending serves as a crucial poverty alleviation mechanism, providing accessible income generation opportunities for individuals with limited capital, education, or formal sector employment prospects. Income studies across multiple countries reveal significant variation in earnings based on location, product specialization, operating hours, and market conditions, with daily earnings typically ranging from $2-15 USD equivalent, placing many vendors above extreme poverty thresholds while remaining economically vulnerable (Steyn *et al.,* 2014). In Nigerian contexts, detailed income analysis by Onyenekenwa (2011) found average daily earnings of ₦2,500-8,000 ($6-20 USD at historical exchange rates), with higher earnings concentrated in urban centers and premium locations.

The low barrier to entry represents a fundamental advantage of street food vending for poverty alleviation, requiring minimal initial capital investment typically ranging from $10-100 USD for basic equipment and initial inventory. This accessibility enables individuals from extremely poor households to initiate income-generating activities without extensive savings, credit access, or formal business registration requirements (Mitullah, 2003). Capital requirements remain manageable through incremental business development, equipment rental arrangements, and supplier credit systems that enable gradual business expansion without substantial upfront investment.

Flexible income generation patterns allow street food vendors to adapt working hours, product offerings, and locations based on household circumstances, market conditions, and seasonal variations. This flexibility proves particularly important for women managing domestic responsibilities, individuals with health constraints, and households requiring income diversification strategies (Sinha, 2005). The ability to scale operations up or down based on circumstances provides important economic resilience and adaptation capabilities that formal employment often lacks.

Risk management and income smoothing functions of street food vending contribute to household economic stability through diversified income sources, flexible operations, and community-based support networks. Many vendors operate street food businesses alongside other economic activities, creating diversified livelihood portfolios that reduce vulnerability to individual sector shocks (Rakodi, 2002). Seasonal adjustments, product diversification, and location flexibility enable vendors to maintain income flows despite market fluctuations, weather variations, and economic disruptions.

Savings and investment patterns among street food vendors demonstrate significant capital accumulation capabilities that contribute to asset building, business expansion, and intergenerational wealth transfer. Studies indicate that successful vendors typically save 15-30% of gross earnings, with savings used for business expansion, education investments, housing improvements, and emergency reserves (Cross, 2000). Access to informal savings mechanisms, including rotating savings and credit associations (ROSCAs), enables collective capital mobilization that supports individual business development and community economic advancement.

2.4.3 Food Security and Nutrition Contributions

Street food systems play crucial roles in urban food security by providing accessible, affordable nutrition to diverse population segments while connecting rural agricultural production with urban consumption patterns. Food accessibility analysis reveals that street foods are typically available within walking distance for most urban residents, operating in high-density neighborhoods, transportation hubs, workplace areas, and educational institutions where formal food retail options are limited (Crush and Frayne, 2011). This spatial accessibility proves particularly important for low-income populations residing in informal settlements or peripheral urban areas with limited commercial food infrastructure.

Affordability comparisons consistently demonstrate that street foods cost 30-60% less than equivalent restaurant meals and 15-40% less than home cooking when opportunity costs of preparation time are included. This cost advantage makes street foods essential food sources for urban poor households allocating 60-80% of income to food expenditures (Maxwell *et al.,* 2000). Price accessibility enables caloric intake maintenance during economic stress periods and provides affordable protein, micronutrient, and energy sources for vulnerable populations including laborers, students, and informal workers.

Nutritional contribution assessments reveal complex profiles combining both positive contributions and concerning deficiencies that require nuanced understanding rather than simplistic categorization. Positive aspects include significant caloric provision (often 20-40% of daily energy intake for regular consumers), accessible protein sources through meat, fish, and legume-based preparations, and culturally appropriate foods that maintain dietary preferences and traditional nutritional knowledge (Steyn *et al.,* 2014). However, concerns include high

sodium content, excessive oil use, limited vegetable incorporation, and micronutrient deficiencies that require targeted improvement interventions.

Cultural nutrition preservation represents an important but often overlooked contribution of street food systems to food security and cultural identity maintenance. Traditional street foods maintain indigenous ingredients, preparation methods, and nutritional knowledge that support dietary diversity and cultural continuity in urban environments (Oyewole, 1997). This cultural dimension of food security extends beyond caloric provision to encompass identity, social cohesion, and traditional ecological knowledge preservation that contributes to overall community well-being and resilience.

Food system resilience contributions of street foods demonstrate particular importance during crisis periods including economic downturns, natural disasters, and health emergencies when formal food systems face disruption. Street food vendors demonstrated remarkable adaptability during COVID-19 pandemic restrictions, implementing delivery systems, adjusting product offerings, and maintaining food access when restaurants and formal food retailers faced closures (Reardon *et al.,* 2021). This crisis resilience highlights street food systems' importance for emergency food security and community survival strategies.

2.4.4 Economic Development and Market Integration

Street food sectors contribute to broader economic development through market integration, value chain development, local economic stimulation, and entrepreneurship promotion that extends benefits beyond individual vendor incomes to encompass community and regional economic advancement. Market integration functions connect smallholder farmers with urban

consumers through direct procurement relationships, creating market access opportunities for rural producers while ensuring fresh, locally sourced ingredients for urban food systems (Reardon and Gulati, 2008). These rural-urban linkages stimulate agricultural production, support rural livelihoods, and contribute to regional economic integration.

Value chain development within street food sectors creates opportunities for food processing, packaging, equipment manufacturing, and distribution businesses that serve vendor needs while generating additional employment and economic activity. Local food processing enterprises emerge to supply street food vendors with partially prepared ingredients, specialized seasonings, packaging materials, and prepared components that enable vendors to focus on final preparation and customer service (Tinker, 1997). Equipment manufacturing and repair services develop specialized products and services adapted to street food operational requirements, creating technical innovation and manufacturing employment.

Local economic multiplier effects result from street food vendor spending patterns that predominantly support local suppliers, service providers, and community businesses. Economic impact studies indicate that 70-85% of street food vendor expenditures remain within local economies through ingredient purchases, equipment acquisition, service payments, and household consumption, creating substantial local economic stimulation effects (Bromley, 2000). These local spending patterns contribute to community economic development and support broader informal sector ecosystem development.

Entrepreneurship development and business skill formation within street food sectors create human capital that supports broader economic development objectives. Successful street food vendors develop sophisticated business management, customer service, financial planning, and

marketing capabilities that are transferable to other economic activities and contribute to overall entrepreneurship capacity within communities (Cross, 2000). Many street food vendors subsequently establish more formal businesses, employ additional workers, and contribute to economic diversification and development.

Innovation and adaptation capabilities demonstrated within street food sectors contribute to economic resilience and competitive advantage development. Street food vendors continuously innovate in product development, service delivery, cost management, and customer engagement, creating competitive pressures and innovation incentives that drive broader economic efficiency improvements (Hart, 1973). Technology adoption, business model innovation, and market adaptation strategies developed within street food sectors often diffuse to other economic sectors and contribute to overall economic modernization.

2.4.5 Social Capital Formation and Community Development

Street food vending contributes significantly to social capital formation through network development, community organization, mutual support systems, and collective action capabilities that strengthen community resilience and social cohesion. Vendor networks create extensive social connections that facilitate information sharing, collaborative problem-solving, mutual assistance, and collective advocacy for improved operating conditions and policy support (Lyons and Brown, 2010). These networks extend beyond individual business interests to encompass community welfare, conflict resolution, and social protection functions that contribute to overall community well-being.

Community organization development emerges from street food vendor associations, informal groups, and collective initiatives that address common challenges including regulatory issues, infrastructure needs, security concerns, and market development opportunities. These organizations develop governance structures, leadership capabilities, and advocacy skills that contribute to broader community development and democratic participation (Mitullah, 2003). Vendor associations often evolve into broader community development organizations addressing housing, education, health, and infrastructure needs that benefit entire communities.

Social protection functions provided through street food vendor networks include emergency assistance, health support, childcare cooperation, and crisis response capabilities that substitute for limited formal social protection systems. Informal insurance mechanisms, collective risk-sharing arrangements, and mutual aid systems provide essential social security functions for vendor families and broader community members (Sethuraman, 1998). These social protection functions prove particularly important for women vendors managing household responsibilities and community care obligations.

Cultural preservation and transmission activities associated with street food vending maintain traditional knowledge, cultural practices, and intergenerational learning that contribute to cultural continuity and identity preservation.

Traditional food preparation techniques, cultural celebrations, and community gathering functions associated with street food activities maintain cultural connections and provide spaces for cultural transmission and social integration (Brown, 2006). This cultural dimension contributes to community identity, social cohesion, and cultural resilience in rapidly changing urban environments.

Conflict resolution and community mediation functions develop within street food vendor networks through established dispute resolution mechanisms, leadership structures, and community negotiation processes. These capabilities contribute to community peace-building, social stability, and collaborative governance that extends beyond vendor communities to influence broader neighborhood and urban governance systems (Roever, 2014). The development of informal governance capabilities strengthens community resilience and democratic participation.

2.4.6 Urban Development and Spatial Economics

Street food vending contributes to urban development through place-making, economic activation of public spaces, transportation hub development, and neighborhood economic vitality that influences broader urban planning and development outcomes. Economic activation functions transform underutilized public spaces, vacant lots, transportation corridors, and marginal urban areas into economically productive and socially active spaces that contribute to overall urban vitality and economic development (Cross, 2000). This spatial activation creates economic value, enhances public safety through increased activity, and contributes to neighborhood development and property value improvement.

Transportation hub integration demonstrates street food vending's role in supporting urban mobility systems through convenient food provision for commuters, transportation workers, and travelers while creating economic opportunities around transportation infrastructure. Bus stations, train terminals, taxi stands, and informal transportation hubs develop street food clusters that serve transportation-related populations while creating employment opportunities and supporting transportation system functionality (Bhowmik, 2005). This integration contributes to

transportation system efficiency and user convenience while generating economic activity around public infrastructure investments.

Neighborhood economic development results from street food clusters that attract customers, generate foot traffic, and support complementary businesses including retail, services, and entertainment activities. Street food concentrations often serve as economic anchors that stimulate broader neighborhood commercial development and property value appreciation (Bromley, 2000). The clustering effects create economic agglomeration benefits that support business development, innovation, and competitive advantage within local markets.

Public space utilization patterns demonstrate street food vending's role in creating active, safe, and socially engaged public spaces that contribute to overall urban quality of life and community well-being. Street food activities generate pedestrian traffic, social interaction, and community surveillance that enhances public safety while creating opportunities for social mixing, cultural exchange, and community building (Mitchell, 2003). These social activation functions contribute to inclusive public space development and community cohesion in diverse urban environments.

Infrastructure development needs and opportunities created by street food concentrations influence urban planning priorities and investment decisions while creating opportunities for public-private partnerships and inclusive urban development approaches. Successful street food districts require infrastructure investments including water supply, sanitation, waste management, electricity, and transportation access that benefit broader communities while supporting vendor operations (Mitullah, 2003).

These infrastructure development opportunities create possibilities for inclusive urban development that addresses informal sector needs while improving overall urban infrastructure and services.

2.5 Significance of Street-Vended Foods

The Significance of Street Vended Foods

Street vended foods represent a critical component of urban food systems worldwide, serving multifaceted roles that extend far beyond simple nutrition provision. These informal food enterprises contribute significantly to food security, particularly in developing nations where they provide affordable, accessible meals to millions of low-income urban residents who lack time or facilities for home cooking (FAO, 2020). The economic significance is profound, street food vending creates substantial employment opportunities, particularly for women, migrants, and marginalized populations who face barriers to formal sector employment, while requiring minimal capital investment and operating outside restrictive regulatory frameworks (Steyn *et al.,* 2014). According to the Food and Agriculture Organization (2007), approximately 2.5 billion people worldwide consume street food daily, representing a massive global industry that generates billions of dollars in economic activity while remaining largely informal and unregulated. From a cultural perspective, street foods preserve traditional culinary heritage, regional flavors, and indigenous cooking techniques that might otherwise disappear in increasingly globalized food landscapes (Cohen and Garrett, 2010). They serve as living repositories of gastronomic diversity, offering authentic taste experiences that reflect local identities and historical food practices. The social function of street food is equally important, as vending sites become informal community gathering spaces that facilitate social interaction,

cultural exchange, and urban vibrancy (Bhowmik, 2012). Recent scholarship has highlighted how street food contributes to urban tourism economies, with food tours and culinary experiences centered on street vendors becoming major attractions in cities globally, transforming street food from survival strategy to celebrated cultural phenomenon (Hiamey and Amenumey, 2013). However, the sector faces ongoing challenges regarding food safety standards, hygiene practices, and regulatory recognition, as vendors often operate in legal gray zones without access to proper infrastructure, training, or health oversight (Samapundo *et al.,* 2016). Studies have documented various foodborne pathogens and contamination risks associated with street vended foods, including bacterial pathogens like *Salmonella, E. coli, and Staphylococcus aureus,* raising legitimate public health concerns that require systematic interventions rather than punitive approaches (Ma *et al.,* 2019).

Despite persistent concerns about safety and sanitation, contemporary research increasingly recognizes street vended foods as legitimate and valuable elements of sustainable urban development. Studies demonstrate that when properly regulated and supported with appropriate infrastructure including designated vending zones, access to clean water, waste disposal systems, and food safety training street food operations can meet acceptable hygiene standards while maintaining their economic and cultural benefits (Rane, 2011.,). The COVID-19 pandemic dramatically impacted the sector, exposing the vulnerability of informal food workers while simultaneously highlighting their resilience and essential role in urban food security during crisis periods, with many vendors adapting rapidly to implement hygiene protocols and contactless service models (Resnick, 2020). Current policy discussions emphasize the need for inclusive approaches that formalize street vending without destroying its inherent flexibility and

accessibility, advocating for collaborative governance models that involve vendors in decision-making processes and recognize their rights to public space and livelihood security.

2.6 Microbial contamination of street vended foods

Street-vended foods in Nigeria, encompassing a vibrant array of ready-to-eat (RTE) items such as suya (grilled skewered meat), akara (fried bean cakes), moi moi (steamed bean pudding), jollof rice, roasted fish, shawarma, African salads (onugbu or abacha with utara), puffed snacks like kokoro, and beverages like zobo (hibiscus drink) and kunun-zaki (millet-based porridge), constitute a cornerstone of urban nutrition and economy, providing affordable, convenient, and culturally embedded sustenance to over 70% of low-income urban dwellers in cities like Lagos, Abuja, Kano, Enugu, Owerri, and Zaria, while supporting livelihoods for millions of informal vendors predominantly women who operate from mobile carts, roadside stalls, or open markets amid rapid urbanization and economic pressures that limit access to formal food systems (Omotosho *et al ., 2023*., Grace *et al ., 2022*)., however, the informal, unregulated nature of SVF vending exposes these foods to pervasive microbial contamination at every stage from raw material sourcing to preparation, storage, and service primarily due to contaminated water sources harboring fecal coliforms (e.g., >1,000 CFU/100 mL in irrigation water used for vegetables), poor personal hygiene among vendors (with only 31-48% washing hands with soap or utensils with clean water, and 100% handling money and food bare-handed in Owerri), inadequate sanitation at vending sites (e.g., open waste bins attracting flies and rodents, improper sewage disposal in flood-prone areas), environmental exposures like dust, insects (Musca domestica carrying up to 10^6 CFU of pathogens), and high ambient temperatures (>30°C) enabling rapid bacterial growth via time-temperature abuse (e.g., foods displayed >4 hours without refrigeration leading to >10^5 CFU/g increases), cross-contamination from shared

unsanitized cutting boards (used for raw meat and RTE salads in 73% of Lagos vendors), and biofilms on utensils perpetuating pathogens, as evidenced by studies in Lagos (high microbial loads in Apapa markets from poor waste disposal), Kano (unsatisfactory coliform counts in Bichi Central Market samples), and Rivers State (TSC 6.00-8.00 log10 CFU/g in rainy-season roasted fish) (Israel & Samuel, 2020., Bichi & Hamza, 2024., Oyet *et al.,* 2020., Chukuezi, 2010)., these vulnerabilities result in alarmingly high prevalence of bacterial pathogens, including *Escherichia coli* (20-35% in salads and rice, often enteroaggregative or Shiga-toxin producing strains indicating fecal contamination), *Staphylococcus aureus* (7.5-33%, with 15.9-31% coagulase-positive isolates producing enterotoxin A in Zaria and Enugu samples, linked to nasal/skin carriage in 40% of vendors), *Salmonella spp*. (7.5-30%, causing typhoid and gastroenteritis in Azikoro and Lagos outbreaks), *Shigella spp*. (up to 34.4% in vegetable-based foods like African salads), *Klebsiella pneumoniae* (30.8%, ESBL-producing in 34% of RTE meats), *Bacillus cereus* (17.5-26.3%, spore-formers in puffed snacks and rice causing emetic/diarrheal syndromes), *Vibrio parahaemolyticus and V. cholerae* (detected in 24% of southern salads via TCBS agar, with serogroups O1-O139 posing *cholera* risks in humid coastal areas), *Proteus mirabilis* (12.5%), *Enterobacter aerogenes* (2.5%), *Citrobacter freundii* (5%), *Pseudomonas aeruginosa* (7.7%), *Yersinia enterocolitica* (7.5%), and emerging opportunists like *Acinetobacter baumannii* and *Kurthia gibsonii* in all RTE categories, alongside total aerobic plate counts (TAPC) exceeding safe limits (>10^5 CFU/g in 34.2% of samples), *Enterobacteriaceae* at 11.3-33%, and coliforms in 69% of beverages, as quantified in cross-sectional analyses using streak-plate, VITEK, VIDAS immunoassays, and PCR for resistance genes (blaCTX-M, mecA, tetA) (Zige and Ayibakeme, 2023., Igbinosa *et al.,* 2021., Smith *et al.,* 2025., Adeyemi *et al.,* 2023., Oluwafemi *et al.,* 2024.,)., mycotoxins like aflatoxins and fumonisins further compound risks in

swallow meals (e.g., eba, fufu) from contaminated raw grains, with two reported outbreaks 2023-2024 linking *dysentery* to *Salmonella/Shigella* in Enugu salads (Afolabi *et al.,* 2024., Beshiru *et al.,* 2020)., health impacts are profound, manifesting as acute gastroenteritis (diarrhea, vomiting within 2-72 hours from *staphylococcal or bacillary toxins), cholera (Vibrio*-linked in southern Nigeria), typhoid fever (*Salmonella* in 15% of Bayelsa rice/beans), hemolytic uremic syndrome (STEC *E. coli*), listeriosis (though less prevalent, up to 20% mortality in vulnerable groups), and chronic sequelae like reactive arthritis or Guillain-Barré from *Campylobacter* (rare but noted in poultry suya), contributing to Nigeria's staggering 200,000+ annual foodborne deaths 40% urban-linked and $15-110 billion economic losses from hospitalizations and productivity dips, disproportionately affecting children under five (125,000 diarrhea deaths/year), pregnant women, and immunocompromised individuals in low-income settlements where SVFs supply 40% of calories (Grace *et al.,* 2022)., antimicrobial resistance (AMR) escalates this crisis, with 50-70% of isolates MDR to β-lactams (ampicillin, penicillin), 40% MRSA in Delhi-inspired Nigerian strains, ESBL/CRE in 34% of *Klebsiella/ E. coli* from Arba Minch-analogous Lagos markets, and 54 resistance genes (e.g., blaTEM) detected via WGS in 2024 scoping reviews, positioning SVFs as urban reservoirs for horizontal gene transfer via biofilms and fecal-oral routes, driven by OTC antibiotic misuse in livestock and poor sanitation (Odetokun *et al .,* 2023., Asfaw *et al .,* 2025., Nwankwo *et al .,* 2025)., socioeconomic amplifiers include poverty (60% vendors lack piped water), informal sector evasion of NAFDAC/SON regulations (<10% licensed in Kano), urban density (52% contamination in high-traffic Abuja sites), climate change (flooding boosting *Vibrio* in 2025 rainy seasons), and knowledge gaps (41.7% vendors misidentifying microbial causes in Osun State), as per 2023-2025 reviews (Kamau *et al.,* 2025., Aluh & Aluh, 2020., Okuyelu *et al.,* 2024)., mitigation hinges on multifaceted, One Health interventions, vendor

HACCP training (30% contamination drop in Oyo pilots via FAO modules), biannual health screenings enforced in Lagos (reducing *Listeria* <10%), infrastructure upgrades like solar refrigeration and potable water stations (96% handwashing compliance post-intervention), rapid kits (ISO 4833-1,2013 for TAPC, VIDAS for *Salmonella*), probiotic enhancements in fermented akara to inhibit pathogens, consumer apps for hygiene ratings, and policy collaborations between NAFDAC, local governments, and NGOs for genomic surveillance and waste management, as advocated in 2024-2025 frameworks to transform SVFs from toxic temptations to safe staples without undermining their socioeconomic lifeline.

2 .6. 1 Sources of Microbial contamination

Microbial contamination represents a pervasive and multifaceted challenge across food production, pharmaceutical manufacturing, healthcare environments, water treatment systems, and everyday human activities, arising from an intricate web of natural reservoirs, ecological cycles, and anthropogenic interventions that facilitate the introduction, survival, and proliferation of bacteria, viruses, fungi, protozoa, and their resilient forms such as spores, cysts, or biofilms. In the atmosphere, microbial aerosols are generated through natural processes like sea spray (releasing *Vibrio spp., Photobacterium*), soil resuspension (carrying *Bacillus anthracis* spores, *Coccidioides immitis arthroconidia*), volcanic emissions, and plant decomposition, while anthropogenic activities combustion engines, agricultural tilling, wastewater aeration, and construction elevate particulate-bound microbes including *Legionella, Mycobacterium, Aspergillus fumigatus, and Penicillium spp*. into air currents, enabling long-range transport and deposition onto food surfaces, medical devices, or cleanroom environments (Amato *et al.,* 2017). Water systems serve as critical conduits and amplifiers of contamination, surface waters receive runoff laden with *Cryptosporidium parvum oocysts, Giardia lamblia cysts, Salmonella*

enterica, and Escherichia coli from agricultural fields fertilized with untreated manure or grazed by livestock, while groundwater may harbor indigenous iron- and sulfur-oxidizing bacteria *(Gallionella, Thiobacillus)* or pathogenic infiltrates via septic leakage., municipal distribution networks foster *Pseudomonas aeruginosa, Acinetobacter baumannii, and Mycobacterium avium* complex in biofilms on pipe walls, especially under low flow or warm conditions, and cooling towers aerosolize *Legionella pneumophila* serogroup 1, responsible for outbreaks in hospitals and hotels (Wingender & Flemming, 2011). Soil and agricultural ecosystems constitute primary reservoirs, enteric pathogens *(Campylobacter jejuni, Listeria monocytogenes, Salmonella Typhimurium DT104)* persist in fecal deposits from wildlife, livestock, or birds, surviving months in moist, organic-rich matrices and transferring to crops via splash during rainfall, direct root uptake, or irrigation with contaminated water., fungal pathogens like *Fusarium spp. and Rhizoctonia* solani overwinter in soil debris, while spore-forming *Clostridium botulinum and Bacillus cereus* thrive in anaerobic microsites, posing risks to canned or vacuum-packed foods (Islam *et al.,* 2004). Human and animal vectors are dynamic sources, healthcare workers' hands transiently carry *Staphylococcus aureus* (including MRSA), *Clostridium* difficile spores, or *Klebsiella pneumoniae* carbapenemase-producers, transferring them to patients via catheters, surgical sites, or ventilators., food handlers shed norovirus (genogroups I/II), hepatitis A, or *Shigella* through fecal-oral routes during inadequate handwashing post-toilet use., livestock and pets harbor zoonotic agents (*Brucella, Coxiella burnetii, Toxoplasma gondii*) transmissible via unpasteurized milk, undercooked meat, or direct contact (Todd *et al.,* 2008,). Food and feed supply chains experience intrinsic contamination *Bacillus cereus* emetic strains in rice starch, *Clostridium perfringens* in raw poultry*, Staphylococcus aureus* enterotoxins in cream-filled pastries and extrinsic cross-contamination from knives, cutting boards, drip from raw meat onto

ready-to-eat salads, or pest vectors, cockroaches mechanically transporting *Salmonella* on legs, rodents depositing *Yersinia enterocolitica* via urine, and houseflies regurgitating *Campylobacter* onto exposed produce (Oyarzabal, 2012,).Industrial and pharmaceutical settings introduce unique risks, purified water systems in drug manufacturing develop Ralstonia pickettii, Burkholderia cepacia complex, or Sphingomonas biofilms resistant to sanitizers., cleanrooms suffer particle ingress of human skin squames carrying *Micrococcus and Corynebacterium*., cosmetic emulsions support Pseudomonas growth due to contaminated raw materials or inadequate preservation., and bioprocessing fermenters risk phage contamination of bacterial cultures or mycoplasma in cell lines (Sandle, 2015). Healthcare environments amplify nosocomial spread, high-touch surfaces (bedrails, keyboards) retain *Acinetobacter baumannii* in dry biofilms, laundry processes re-aerosolize C. difficile, and endoscopes harbor *Mycobacterium* chimaera in heater-cooler units due to water stagnation (Dancer, 2014). Finally, global trade and climate change exacerbate risks imported spices carry *Salmonella* Rissen, frozen berries transmit hepatitis A, and warming temperatures expand vector ranges for *Vibrio vulnificus* in coastal waters illustrating how interconnected ecological, behavioral, and technological factors converge to sustain microbial contamination cycles that demand rigorous, multi-barrier prevention strategies encompassing source control, sanitation, monitoring, and education (Yeni *et al.,* 2016)

2.7 Common food borne pathogens in street vended foods

Street-vended foods (SVFs) in Nigeria, encompassing popular items like suya (grilled meat), akara (bean cakes), moi moi, jollof rice, puff-puff, fufu with soups, garri, and roasted plantain (boli), serve as critical sources of nutrition and income for urban and peri-urban populations, yet

they are major vehicles for foodborne pathogens due to systemic deficiencies in hygiene, infrastructure, and regulatory oversight. The predominant bacterial pathogens include *Escherichia coli* (particularly pathogenic strains such as enteropathogenic *E. coli* [EPEC], enterotoxigenic *E. coli* [ETEC], and enterohemorrhagic *E. coli* [EHEC] serotype O157,H7), which contaminate foods via fecal-oral routes from contaminated water, unwashed vegetables, or poor hand hygiene, with studies in Kano showing 62.5% of ready-to-eat salads harboring *E. coli* above permissible limits (Onyeneho and Hedberg, 2013). *Salmonella* species, notably S. Typhi and non-typhoidal *Salmonella* (e.g*., S. Enteritidis, S. Typhimurium*), are frequently isolated from undercooked poultry, eggs, and meat-based SVFs, with a cross-sectional study in Ibadan detecting *Salmonella* in 28% of suya samples and linking outbreaks to improper cooking temperatures below 75°C (Smith *et al.,* 2016). *Shigella* spp. (S. flexneri, S. sonnei, S. dysenteriae) thrive in moist, carbohydrate-rich foods like rice and beans left at ambient temperatures, contributing to bacillary dysentery., research in Enugu revealed 18.4% prevalence in moi moi and attributed transmission to vendors not washing hands after defecation (Oranusi & Olorunmowaju, 2014). Staphylococcus aureus, a ubiquitous skin and nasal colonizer, produces heat-stable enterotoxins in foods held between 7–60°C (the "danger zone"), with 45.7% of puff-puff and meat pie samples in Lagos exceeding 10^5 CFU/g due to bare-hand contact and prolonged display without temperature control (Adeyeye, 2018). *Bacillus cereus*, both emetic (from preformed cereulide in fried rice) and diarrheal (from enterotoxins produced in the gut after spore germination in starchy foods), was identified in 22% of reheated jollof rice in Abuja, often from vendors reusing oil and storing cooked rice overnight without refrigeration (Ogbonna *et al.,* 2020). Clostridium perfringens type A, a spore-forming anaerobe, causes "cafeteria poisoning" in bulk-prepared stews and soups left cooling slowly, with outbreaks traced to egusi

and okro soups in Ogun State (Akinyemi *et al.,* 2019). *Campylobacter jejuni*, though understudied in Nigeria due to diagnostic limitations, emerges in poultry-based SVFs like grilled chicken, with molecular surveys in Jos detecting thermophilic *Campylobacter* in 35% of raw and 12% of cooked samples, surviving due to inadequate internal cooking to 74°C (Ngotho *et al.,* 2021). Beyond bacteria, parasitic pathogens such as *Entamoeba histolytica, Giardia lamblia*, and *Cryptosporidium parvum* contaminate salads and fruit juices washed with untreated water, while Ascaris lumbricoides eggs persist on unwashed vegetables sold alongside SVFs (Okojie & Isah, 2014). Fungal contaminants like *Aspergillus flavus* producing aflatoxin B1 are common in groundnut-based snacks and kulikuli, with levels up to 120 µg/kg in northern Nigeria, far exceeding the 4 µg/kg EU limit, posing hepatocarcinogenic risks (Ezekiel *et al.,* 2018). Contamination pathways include, (1) pre-harvest (animal feces, contaminated irrigation water)., (2) processing (use of untreated borehole or sachet water only 12% of vendors in Benin City use potable water [Eromo *et al.,* 2019])., (3) handling (78% of vendors handle money and food interchangeably without handwashing [Ifeadike *et al.,* 2015])., (4) storage/display (foods kept >4 hours at 30–40°C ambient temperature)., and (5) environmental exposure (vending near open drains, refuse dumps, and heavy traffic, facilitating fly-borne transfer). A meta-analysis of 47 Nigerian studies (2000–2023) reported pooled prevalence of 41.2% for coliforms, 29.8% for S. aureus, and 19.6% for *Salmonella* in SVFs, with northern regions showing higher contamination due to arid dust and limited water access (Ibrahim *et al.,* 2023). The public health impact is severe, Nigeria records ~150 million cases of acute gastroenteritis annually, with 20–30% attributed to SVFs, causing 35,000–50,000 deaths, mostly in children under five, and economic losses exceeding NGN 110 billion yearly in healthcare and productivity (WHO, 2019., Fowora *et al .,* 2021). Multi-drug resistance is rampant—80% of *Salmonella* isolates from SVFs in Lagos

were resistant to ampicillin, tetracycline, and cotrimoxazole (Olosunde *et al.,* 2020) complicating treatment and increasing mortality. Mitigation requires integrated interventions, mandatory food handler certification (currently only 9% trained [Nwaokoro *et al.,* 2017]), solar-powered refrigeration units, point-of-use water treatment (e.g., chlorination), color-coded utensils to prevent cross-contamination, and real-time microbial testing using affordable rapid kits, as piloted in Oyo State with 60% reduction in coliform loads post-intervention (Adebayo-Oyetoro *et al.,* 2022).

2.7.1 Escherichia coli

Escherichia coli represents one of the most extensively studied and multifaceted bacterial species in microbiology, serving simultaneously as a harmless commensal inhabitant of the gastrointestinal tract, a valuable model organism for scientific research, and a significant human pathogen responsible for substantial morbidity and mortality worldwide. First isolated and characterized by German-Austrian pediatrician Theodor Escherich in 1885 from the fecal samples of healthy infants, this gram-negative, facultative anaerobic bacterium belongs to the family Enterobacteriaceae and exhibits remarkable genetic and phenotypic diversity across its numerous strains and serotypes. Morphologically, *E. coli* presents as a rod-shaped (*bacillus*) bacterium typically measuring 2.0 micrometers in length and 0.25-1.0 micrometers in diameter, possessing a complex cell envelope structure characteristic of gram-negative bacteria, consisting of an inner cytoplasmic membrane, a thin peptidoglycan layer within the periplasmic space, and an outer membrane rich in lipopolysaccharides (LPS) that serves as an endotoxin and contributes to the bacterium's pathogenic potential in certain strains. Most *E. coli* strains exhibit motility

through peritrichous flagella multiple flagella distributed around the entire cell surface though non-motile variants naturally occur and some pathogenic strains have evolved to lose motility as part of their adaptive strategies. The bacterial genome typically comprises a single circular chromosome of approximately 4.6 million base pairs encoding roughly 4,300 genes, though considerable genomic plasticity exists among different strains, with substantial variations in gene content that can range from 4.0 to 5.5 million base pairs depending on the strain's evolutionary history and ecological adaptation (Kaper *et al.,* 2004., Lukjancenko *et al.,* 2010., Tenaillon *et al.,* 2010). This genomic flexibility is facilitated by horizontal gene transfer mechanisms including conjugation, transduction, and transformation, which enable *E. coli* to acquire virulence factors, antibiotic resistance genes, and metabolic capabilities encoded on plasmids, bacteriophages, transposons, and chromosomal pathogenicity islands. *E. coli*'s metabolic versatility is remarkable, as it can grow under both aerobic and anaerobic conditions, ferment various sugars including glucose, lactose, and other carbohydrates, and synthesize all necessary cellular components from simple carbon and nitrogen sources, making it highly adaptable to diverse environmental conditions. In its primary ecological niche the lower gastrointestinal tract of warm-blooded animals. *E. coli* typically represents less than 1% of the total intestinal microbiota by number, yet it plays disproportionately important roles in host physiology despite being a minority member of this complex microbial community. These commensal *E. coli* strains colonize the intestinal mucus layer within hours to days after birth and establish lifelong symbiotic relationships with their hosts, contributing to several beneficial functions including the synthesis of vitamin K2 (menaquinone), which is essential for blood coagulation and bone metabolism., production of antimicrobial compounds such as colicins that inhibit colonization by pathogenic bacteria through competitive exclusion., stimulation and maturation of the host

immune system through continuous low-level antigenic stimulation that helps calibrate appropriate immune responses., and participation in the digestion and metabolism of complex carbohydrates and other nutrients that escape absorption in the small intestine (Tenaillon *et al.,* 2010., Croxen *et al.,* 2013).

However, *E. coli*'s remarkable genomic plasticity and capacity for horizontal gene acquisition have enabled the evolution of numerous pathogenic variants (pathotypes) that have acquired specific virulence factor combinations, transforming this normally benign commensal into a versatile pathogen capable of causing diseases ranging from self-limiting gastroenteritis to life-threatening systemic infections. The pathogenic *E. coli* strains are classified into distinct pathotypes based on their virulence mechanisms, clinical presentations, and epidemiological characteristics, enterotoxigenic *E. coli* (ETEC), which produces heat-labile and/or heat-stable enterotoxins and is a leading cause of traveler's diarrhea and childhood diarrhea in developing countries, causing an estimated 200 million cases and 380,000 deaths annually, primarily in children under five years of age., enteropathogenic *E. coli* (EPEC), historically the first *E. coli* pathotype identified as a cause of infant diarrhea in the 1940s-1950s, which causes attaching and effacing lesions on intestinal epithelial cells through the formation of characteristic pedestal structures., enterohemorrhagic *E. coli* (EHEC), also known as Shiga toxin-producing *E. coli* (STEC), with serotype O157,H7 being the most notorious member, which produces potent cytotoxins (Shiga toxins Stx1 and Stx2) that can cause hemorrhagic colitis characterized by bloody diarrhea and, in approximately 10-15% of cases, progress to hemolytic uremic syndrome (HUS), a potentially fatal complication involving acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia, with children under five and elderly adults being particularly vulnerable., enteroinvasive *E. coli* (EIEC), which shares pathogenic mechanisms

with *Shigella* species and invades and destroys colonic epithelial cells, causing dysentery-like illness with fever, abdominal cramps, and bloody diarrhea., enteroaggregative *E. coli* (EAEC), characterized by its distinctive "stacked brick" aggregative adherence pattern to intestinal cells and associated with persistent diarrhea in both children and adults, including in HIV-infected individuals., diffusely adherent *E. coli* (DAEC), which exhibits a diffuse adherence pattern and is associated with diarrhea primarily in children aged 18 months to 5 years., and the extraintestinal pathogenic *E. coli* (ExPEC) group, which includes uropathogenic *E. coli* (UPEC) responsible for approximately 80-90% of community-acquired urinary tract infections (UTIs) and a significant proportion of hospital-acquired UTIs, making them the most common bacterial infections in humans with an estimated 150 million cases annually worldwide., neonatal meningitis *E. coli* (NMEC), which causes approximately 20% of neonatal bacterial meningitis cases with mortality rates of 10-15% and significant neurological sequelae in survivors., and sepsis-associated *E. coli* strains that can cause bacteremia, septic shock, and multi-organ failure, particularly in immunocompromised patients, those with indwelling medical devices, or individuals with disrupted anatomical barriers (Kaper *et al.,* 2004., Croxen *et al.,* 2013., Allocati *et al.,* 2013). The virulence factors employed by these pathogenic strains are diverse and sophisticated, including adhesins (fimbriae and afimbrial adhesins) that mediate specific attachment to host cells., toxins such as Shiga toxins, heat-labile and heat-stable enterotoxins, cytotoxic necrotizing factors, and hemolysin., iron acquisition systems including siderophores that sequester iron from host proteins., capsular polysaccharides that provide protection from phagocytosis and complement-mediated killing., the type III secretion system that directly injects effector proteins into host cells to manipulate cellular functions., invasion factors that enable

bacterial entry into non-phagocytic cells., and biofilm formation capabilities that facilitate persistence on medical devices and in the urinary tract (Kaper *et al.,* 2004).

Beyond its medical significance, *E. coli* has achieved unparalleled importance as a model organism in molecular biology, genetics, and biotechnology, with the laboratory strain K-12 and its derivatives serving as the workhorse for countless scientific discoveries and practical applications since its isolation from a convalescent diphtheria patient in 1922. *E. coli*'s relatively simple genetic system, rapid generation time (doubling approximately every 20 minutes under optimal conditions), ease of cultivation on inexpensive media, well characterized genetics and physiology, and amenability to genetic manipulation have made it the organism of choice for fundamental research and industrial applications. Landmark discoveries using *E. coli* include the elucidation of the genetic code, the mechanisms of DNA replication and gene regulation (including the famous lac operon), the discovery of restriction enzymes and their application in recombinant DNA technology, the development of DNA cloning and expression systems, and the establishment of fundamental principles in bacterial physiology and metabolism. In biotechnology and pharmaceutical industries, genetically engineered *E. coli* strains serve as cellular factories for the production of recombinant proteins, including human insulin (the first FDA-approved recombinant protein therapeutic in 1982), human growth hormone, various vaccines, monoclonal antibody fragments, industrial enzymes, and numerous other biologics worth billions of dollars annually in the global pharmaceutical market. However, the increasing prevalence of antibiotic-resistant *E. coli* strains represents one of the most pressing challenges in contemporary medicine and public health, with resistance mechanisms including extended-spectrum beta-lactamases (ESBLs) that hydrolyze most beta-lactam antibiotics except carbapenems., carbapenemases such as New Delhi metallo-beta-lactamase (NDM) and

Klebsiella pneumoniae carbapenemase (KPC) that confer resistance to virtually all beta-lactam antibiotics including last-resort carbapenems., plasmid-mediated quinolone resistance mechanisms., and multidrug resistance efflux pumps that actively export multiple antibiotic classes from bacterial cells. The emergence and global dissemination of pandemic multidrug-resistant *E. coli* clones, particularly the sequence type 131 (ST131) lineage associated with fluoroquinolone resistance and ESBL production, has created situations where common infections such as UTIs become difficult or impossible to treat with standard oral antibiotics, necessitating hospitalization for intravenous antibiotic therapy and sometimes resulting in treatment failure and mortality (Allocati *et al.,* 2013., Poolman & Wacker, 2016). The epidemiology of *E. coli* infections reflects both its commensal nature and pathogenic potential, with transmission occurring through multiple routes including fecal-oral transmission via contaminated food (particularly undercooked ground beef, raw milk, and fresh produce contaminated with animal feces) and water., person-to-person spread, especially in institutional settings such as daycare centers and nursing homes., contact with animals, particularly cattle and other ruminants that serve as asymptomatic reservoirs for EHEC., and endogenous spread from the patient's own intestinal flora to normally sterile sites, as occurs in UTIs and intra-abdominal infections following intestinal perforation. Prevention and control strategies encompass proper food handling and cooking practices, particularly ensuring ground beef reaches internal temperatures of 71°C (160°F)., implementation of Hazard Analysis and Critical Control Points (HACCP) systems in food production., improved sanitation and hygiene, especially handwashing., prudent antibiotic use in both human medicine and animal agriculture to slow resistance development., surveillance systems to detect outbreaks and monitor resistance trends., development of vaccines, though progress has been limited despite decades of research., and

water treatment to prevent waterborne transmission (Kaper *et al.,* 2004., Croxen *et al.,* 2013., Allocati *et al.,* 2013). Current research frontiers include investigating the complex interactions between commensal and pathogenic *E. coli* strains within the intestinal microbiome., developing rapid diagnostic methods to distinguish pathogenic from non-pathogenic strains and detect antibiotic resistance., exploring phage therapy as an alternative or adjunct to antibiotics., engineering probiotic *E. coli* strains for therapeutic applications., utilizing synthetic biology approaches to create designer *E. coli* strains with novel capabilities., understanding the evolutionary dynamics of virulence and resistance gene acquisition., and developing new antimicrobial strategies including anti-virulence compounds that disarm pathogens without killing them, thereby reducing selection pressure for resistance development (Poolman & Wacker, 2016). In conclusion, *E. coli* exemplifies the complexity of microbe-host relationships, representing a paradigmatic example of how a predominantly beneficial commensal organism can, through evolutionary processes and horizontal gene transfer, give rise to pathogenic variants that pose significant threats to human health, while simultaneously serving as an indispensable tool for scientific research and biotechnological applications that have revolutionized modern biology and medicine.

2.7.2 *Klebsiella* species

Klebsiella is a genus of Gram-negative, non-motile, encapsulated, facultatively anaerobic bacteria belonging to the family Enterobacteriaceae, named after German microbiologist Edwin Klebs, and these bacteria are ubiquitous in nature, found in soil, water, plants, and the mucosal surfaces of mammals, with some species being environmental saprophytes while others are

clinically significant opportunistic pathogens causing a wide range of infections in humans and animals (Podschun and Ullmann, 1998). The genus has undergone significant taxonomic revisions, with currently recognized species including *Klebsiella pneumoniae* (the most clinically significant), K. oxytoca, K. aerogenes (formerly Enterobacter aerogenes), *K. variicola, K. quasipneumoniae, K. michiganensis, K. grimontii, and K. huaxiensis, where K. pnuemoniae* is further divided into subspecies and phylogenetic groups with *K. pnuemoniae* sensu stricto being the predominant pathogen (Holt *et al.,* 2015., Wyres *et al.,* 2020).

Microbiologically, *Klebsiella* species are rod-shaped bacilli measuring approximately 0.3-1.0 × 0.6-6.0 μm with a prominent polysaccharide capsule that gives colonies a mucoid appearance on culture media, and they grow readily on standard laboratory media forming large, mucoid, lactose-fermenting pink colonies on MacConkey agar at optimal temperatures of 37°C, with biochemical properties including variable indole reactions (*K. pnuemoniae* negative, K. oxytoca positive), urease positive, citrate positive, methyl red negative, Voges-Proskauer positive, non-motile (distinguishing them from Enterobacter), oxidase negative, and catalase positive (Podschun and Ullmann, 1998., Turton *et al.,* 2008).

The major virulence factors include the capsular polysaccharide (K antigen) with over 80 different K serotypes identified (K1, K2, K5, K20, K54, and K57 associated with hypervirulent strains) that inhibits phagocytosis and prevents complement activation, lipopolysaccharide (O antigen) endotoxin contributing to septic shock, siderophores (enterobactin, yersiniabactin, aerobactin, salmochelin) for iron acquisition with aerobactin production particularly associated with hypervirulent strains, type 1 and type 3 fimbriae mediating adherence and biofilm formation crucial for colonization, and the ability to form biofilms on medical devices contributing to

device-associated infections and antimicrobial resistance (Paczosa and Mecsas, 2016., Bengoechea and Sa Pessoa, 2019., Martin and Bachman, 2018).

Clinically, classical *K. pnuemoniae* (cKp) typically causes healthcare-associated infections including *pneumoniae* (especially ventilator-associated), urinary tract infections (particularly catheter-associated), bloodstream infections, surgical site infections, and intra-abdominal infections in immunocompromised patients with chronic diseases, alcoholism, or diabetes mellitus, while hypervirulent *K. pnuemoniae* (hvKp) emerged primarily in Asia and causes community-acquired invasive infections including pyogenic liver abscesses, meningitis, endophthalmitis, necrotizing fasciitis, and septic metastatic lesions in previously healthy individuals with high mortality rates (Shon *et al.,* 2013., Russo and Marr, 2019., Catalán-Nájera *et al.,* 2017).

The antimicrobial resistance crisis in *Klebsiella* is alarming, with multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) strains emerging globally, primarily through production of extended-spectrum beta-lactamases (ESBLs) hydrolyzing third-generation cephalosporins, carbapenemases (KPC, NDM, OXA-48, VIM, IMP) conferring resistance to carbapenems as last-resort antibiotics, and additional mechanisms including AmpC beta-lactamases, aminoglycoside-modifying enzymes, fluoroquinolone resistance through mutations in gyrA and parC, and colistin resistance via mcr genes and chromosomal mutations (Navon-Venezia *et al.,* 2017., Pitout *et al.,* 2015., Lee *et al.,* 2016).

Treatment challenges are substantial, with carbapenem-resistant *Klebsiella pneumoniae* (CRKP) designated as a critical priority pathogen by the WHO, requiring combination therapy with agents such as ceftazidime-avibactam, meropenem-vaborbactam, imipenem-relebactam,

cefiderocol, tigecycline, polymyxins, aminoglycosides, and fosfomycin, though therapeutic options remain limited with high failure rates and mortality, while emerging hypervirulent carbapenem-resistant strains represent convergent threats combining high virulence with extensive antimicrobial resistance (Pitout *et al.,* 2015., Russo and Marr, 2019., Harada and Doi, 2018).

Diagnosis involves culture-based methods with identification through biochemical testing, MALDI-TOF mass spectrometry, and molecular methods including PCR for virulence genes (rmpA, rmpA2, iucA, iroB, peg-344) and resistance genes, whole-genome sequencing for epidemiological tracking, and phenotypic antimicrobial susceptibility testing by broth microdilution, disk diffusion, or automated systems, with carbapenemase detection through modified carbapenem inactivation method (mCIM), Carba NP test, or molecular assays (Lee *et al.,* 2016., Harada and Doi, 2018., Turton *et al.,* 2008).

Prevention and control strategies include infection prevention measures with hand hygiene, contact precautions for colonized or infected patients, environmental cleaning, surveillance programs for early detection of resistant strains, antimicrobial stewardship to reduce inappropriate antibiotic use, screening high-risk patients in endemic areas, device bundle implementations to prevent catheter and ventilator-associated infections, and vaccination development currently in research phases targeting capsular polysaccharides and surface proteins (Paczosa and Mecsas, 2016., Lee *et al.,* 2016., Navon-Venezia *et al.,* 2017).

Epidemiologically, *Klebsiella* infections occur worldwide with CRKP endemic in many countries particularly in Asia, Southern Europe, South America, and increasingly in North America and Africa, hvKp traditionally prevalent in Asian countries (Taiwan, China, Korea,

Singapore) but now reported globally, healthcare-associated outbreaks common in intensive care units with transmission via contaminated medical equipment and healthcare workers' hands, and high-risk populations including hospitalized patients, ICU patients, those with invasive devices, immunocompromised individuals, neonates in neonatal intensive care units, and patients with prolonged antibiotic exposure (Holt *et al.,* 2015., Russo and Marr, 2019., Pitout *et al.,* 2015., Wyres *et al.,* 2020).

Emerging concerns include the convergence of hypervirulence and multidrug resistance creating extremely dangerous strains, plasmid-mediated horizontal gene transfer facilitating rapid spread of resistance and virulence determinants across bacterial populations, environmental reservoirs in hospital wastewater and community water supplies serving as potential sources of resistant strains, and the limited pipeline of novel antibiotics with few new agents effective against carbapenem-resistant gram-negative bacteria in development, necessitating urgent global action through enhanced surveillance, infection prevention, antimicrobial stewardship, research into novel therapeutics including bacteriophage therapy and immunotherapeutic approaches, and international collaboration to combat this growing public health threat (Catalán-Nájera *et al.,* 2017., Navon-Venezia *et al.,* 2017., Martin and Bachman, 2018., Wyres *et al.,* 2020).

2.8 Hematological parameters as indicators of health

Hematological parameters serve as fundamental biomarkers for assessing overall health status and diagnosing various pathological conditions in both human and veterinary medicine. Complete blood count (CBC) analysis encompasses multiple indicators including red blood cell (RBC) count, hemoglobin (Hb) concentration, hematocrit (HCT), mean corpuscular volume

(MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC) count with differential, and platelet count. These parameters collectively provide insights into oxygen-carrying capacity, immune system functionality, and hemostatic mechanisms (Jain, 1993). Erythrocyte indices such as RBC count, hemoglobin, and hematocrit are particularly crucial for detecting anemia, polycythemia, and various nutritional deficiencies, while leukocyte profiles reveal inflammatory responses, infections, and immune disorders (Feldman *et al.,* 2000). The platelets and coagulation parameters assess hemostatic function and susceptibility to bleeding or thrombotic disorders. Clinical interpretation of these values must consider factors such as age, sex, physiological state, and species-specific reference ranges to accurately determine health status (Weiss and Wardrop, 2010). The sensitivity of hematological parameters to physiological stress, nutritional status, and disease makes them invaluable diagnostic tools in clinical practice. Deviations from normal ranges can indicate acute or chronic pathological processes affecting various organ systems.

Alterations in erythrocyte parameters may reflect bone marrow dysfunction, hemolytic processes, hemorrhage, or nutritional deficiencies of iron, vitamin B12, or folic acid (Bain *et al.,* 2016). Leukocyte abnormalities, including leukocytosis or leukopenia, provide critical information about infectious processes, inflammatory conditions, immunosuppression, or hematological malignancies (Latimer *et al.,* 2003). Neutrophilia typically indicates bacterial infection or tissue inflammation, while lymphocytosis may suggest viral infections or chronic inflammatory conditions. Eosinophilia often correlates with parasitic infections or allergic reactions, and monocytosis can indicate chronic infections or tissue repair processes (Thrall *et al.,* 2012). Furthermore, red blood cell morphology examination reveals specific abnormalities such as anisocytosis, poikilocytosis, or inclusion bodies that can pinpoint specific disease etiologies or

toxicological exposures. The dynamic nature of these parameters allows for monitoring disease progression, treatment efficacy, and recovery status, making serial hematological assessments essential in managing acute and chronic conditions (Harvey, 2012).

Foodborne pathogens exert significant impacts on hematological parameters through various pathophysiological mechanisms including direct invasion of blood cells, toxin production, immune system activation, and induction of systemic inflammatory responses. Bacterial pathogens such as *Salmonella* spp, *Escherichia coli*, *Listeria monocytogenes*, *Campylobacter jejuni*, and *Staphylococcus aureus* can trigger marked alterations in blood cell counts and indices depending on infection severity and host immune response (Kessel *et al.,* 2001). *Salmonella* infections commonly induce leukopenia during the initial bacteremic phase, followed by leukocytosis with left shift as the immune response intensifies, accompanied by relative lymphopenia and possible thrombocytopenia in severe cases (Hohmann, 2001). *Escherichia coli* O157,H7 and other Shiga toxin-producing strains can cause hemolytic uremic syndrome (HUS), characterized by microangiopathic hemolytic anemia with fragmented red blood cells (schistocytes), thrombocytopenia, and elevated lactate dehydrogenase levels due to intravascular hemolysis (Tarr *et al.,* 2005). The pathogenic mechanism involves Shiga toxins binding to globotriaosylceramide receptors on endothelial cells and erythrocytes, leading to cell damage and activation of the coagulation cascade. *Listeria monocytogenes* infections can produce variable hematological changes including monocytosis, though this finding is not consistent, along with leukocytosis and possible anemia in disseminated disease (Lorber, 1997).

Campylobacter jejuni gastroenteritis may result in elevated WBC counts with predominant neutrophilia, and in severe cases, reactive thrombocytosis during recovery phase or thrombocytopenia during acute infection (Blaser and Engberg, 2008). Staphylococcal food

poisoning caused by enterotoxins typically produces milder hematological changes compared to invasive infections, though toxic shock syndrome can lead to profound leukocytosis, left shift, thrombocytopenia, and coagulopathy (Dinges *et al.,* 2000). *Vibrio species*, particularly *Vibrio cholerae* and *Vibrio vulnificus*, can cause severe dehydration leading to hemoconcentration with elevated hematocrit and hemoglobin levels, while septicemic forms may produce leukocytosis, thrombocytopenia, and disseminated intravascular coagulation (Blake *et al.,* 1979). *Yersinia enterocolitica* infections may manifest with leukocytosis, elevated erythrocyte sedimentation rate, and in chronic cases, anemia of chronic disease (Bottone, 1997). *Clostridium perfringens* type A food poisoning generally causes self-limited gastroenteritis with minimal hematological changes, but necrotizing enteritis can lead to severe leukocytosis, anemia from gastrointestinal bleeding, and thrombocytopenia .

2.9 Oxidative stress markers

Oxidative stress markers serve as quantifiable indicators of cellular damage resulting from an imbalance between the production of reactive oxygen species (ROS) and a biological system's antioxidant capacity. When the generation of highly reactive molecules, such as the superoxide anion and hydrogen peroxide (H_2O_2), overwhelms the protective enzymatic and non-enzymatic defenses, these ROS attack and modify essential macromolecules. The most commonly measured end-products include Malondialdehyde (MDA) and 4-Hydroxynonenal (4-HNE), which are stable products of polyunsaturated fatty acid (lipid) peroxidation in cell membranes., their elevated levels reflect significant cellular membrane damage. Similarly, protein carbonyls and 3-nitrotyrosine indicate oxidative damage to proteins, while the detection of 8-hydroxy-2'-

deoxyguanosine (8-OHdG) is widely used as a key biomarker for oxidative damage to DNA. Changes in the activity of endogenous antioxidant enzymes, such as Superoxide Dismutase (SOD), Catalase, and Glutathione Peroxidase (GPx), also function as markers, reflecting the system's attempts to cope with the increased oxidative load. Foodborne pathogens, such as *Salmonella* and *klebsiella* profoundly affect these oxidative stress markers by triggering a robust immune response in the host, primarily through the activation of phagocytic cells like macrophages and neutrophils. Upon encountering the invading bacteria, these immune cells initiate a process known as the oxidative burst, deliberately generating massive, localized amounts of ROS and reactive nitrogen species (RNS) like nitric oxide (NO) as a critical defense strategy to kill the microbes. While successful in pathogen clearance, this excessive production of cytotoxic species in the infection site causes significant collateral damage to the host's surrounding tissues, leading to inflammation and injury that is reflected in the dramatic increase of host oxidative stress markers like MDA and protein carbonyls. The resulting spike in ROS/RNS production and the subsequent exhaustion of host antioxidant reserves (e.g., reduced levels of glutathione and decreased SOD/Catalase activity) are central to the pathogenesis and severity of foodborne infections. In response to this hostile, pro-oxidant environment created by the host's immune system, foodborne pathogens have evolved sophisticated stress tolerance and defense mechanisms that allow them to survive and propagate, directly influencing the prolonged elevation of host stress markers. The bacteria themselves often express their own potent antioxidant enzymes, such as various forms of Superoxide Dismutase and Catalase, which act to neutralize the host-generated ROS/RNS, a mechanism critical for their virulence and intracellular survival within the host. Furthermore, some pathogens may directly contribute to the host's oxidative load by producing toxins or interfering with host signaling pathways, further

amplifying the redox imbalance. Therefore, the measurable host oxidative stress markers are a direct result of the continuous, complex battle between the host's attempt to kill the pathogen via the oxidative burst and the pathogen's ability to resist and subvert that defense, a dynamic struggle that ultimately dictates the persistence and outcome of the foodborne illness

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of Test Organisms

Bacteria isolated from street vended foods were obtained as test organisms for this study. Organisms were tentatively identified as *Escherichia coli* and *Klebsiellia sp,* based on cultural, morphological and biochemical characteristics.

3.2 Preparation of Materials

Transparent boxes with proper aeration at the top and clean plates meant for housing and feeding of the wistar rats were provided by the department of Animal and Environmental Biology, University of Benin as well as digital scale for weighing the rats. Wood shavings were obtained from a local sawmill at Uselu for comfort and absorption, while feed was also obtained from department of Animal and Environmental Biology, University of Benin. Bottles, syringe, nose mask, cotton wool, hand gloves, petri dishes, marker, masking tape were all obtained from a local pharmacy at Ugbowo, Benin city.

3.3 Ethical Approval

The experimental protocol was reviewed and approved by ethics and research compliance committee of the faculty of veterinary medicine, University of Benin, Benin city. All experimental procedures involving the handling and care of animals complied with internationally accepted ethical guidelines for the use of laboratory animals.

3.4 Experimental Design

The study utilized 25 wistar rats obtained from theDepartment of Anatomy , University of Benin, Benin City. These rats were weighed and separated into five groups, V(control), W1, W2, X1 and X2(experimental), each group comprised of five rats which were kept in a transparent boxes that was well ventilated at the top. The boxes were labelled to differentiate the experimental from the control. Rats in each group were labeled for identification. Using a permanent marker to mark specific body parts, such as the hands, legs and nose. The study involved an acclimatization period of 7 days for the rats upon their arrival at the animal house. The rats were fed once daily with pellet form feed, which was placed in a clean plate, while fresh water was provided in separate plates in each box. The wood shavings were changed three times sun a week and the plates were washed everyday before feeding. The rats were weighed on arrival and after acclimatization, the weight were captured using a digital scale. Following acclimatization stage the experimental rats were infected with known innoculum size of each test organisms. Then was done at 3 day interval. All rats, both in the experimental and control groups, had unrestricted access to food and water throughout the study. Also, proper ventilation and sanitation protocols such as changing their beddings regularly were maintained to ensure a clean and hygienic living environment for the rats. After the experimental groups were infected with the organisms in week two, sacrifices were carried out in weeks three, four, and five. Following sacrifice, blood samples and organs were harvested for analysis.

3.5 Determination of Weight Of Rats

The weight of each rat was determined by transferring the rat into a covered container and placing the container on a digital scale (SF-400A). The weight reading was recorded. This

process was repeated for all rats in both the experimental and control groups. Before weighing each rat, the weight of the empty container was determined. Thereafter the weight of the rat and container was determined. Then, when the rat was placed in the container and weighed, we subtracted the container's weight from the total reading to determine the rat's true weight. The weight of each rat was obtained by subtracting W1 from W2 (W2-W1). (len *et al*.,1996)

3.6 Molecular Identification of The Test Organisms

Molecular identification of the bacterial isolates was carried out to confirm their identity of the test organisms using polymerase chain reaction (PCR) techniques.

3.6.1 Bacteria DNA extraction

Briefly, Single colonies grown on medium were transferred to 1.5 ml of liquid medium and cultures were grown on a shaker for 48 h at 28 ºC. After this period, cultures were centrifuged at 4600g for 5 min. The resulting pellets were resuspended in 520 µl of TE buffer (10 mMTris-HCl, 1mM EDTA, pH 8.0). Fifteen microliters of 20% SDS and 3 µl of Proteinase K (20 mg/ml) were then added. The mixture was incubated for 1 hour at 37 ºC, then 100 µl of 5 M NaCl and 80 µL of a 10% CTAB solution in 0.7 M NaCl were added and votexed. The suspension was incubated for 10 min at 65 ºC and kept on ice for 15 min. An equal volume of chloroform, isoamyl alcohol (24,1) was added, followed by incubation on ice for 5 min and centrifugation at 7200g for 20 min. The aqueous phase was then transferred to a new tube and isopropanol (1, 0.6) was added and DNA precipitated at –20 ºC for 16 h. DNA was collected by centrifugation at 13000g for 10 min, washed with 500 µl of 70% ethanol, air-dried at room temperature for approximately three hours and finally dissolved in 50 µl of TE buffer. (Lanniello *et al*., 2025)

3.6.2 PCR Analysis

PCR sequencing preparation cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25mM MgCl2, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each 27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and - 1525R, 5′-AAGGAGGTGATCCAGCC-3′ primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water 8µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a Pcr profile consisting of an initial denaturation at 94°C for 5 min., followed by a 30 cycles consisting of 94°C for 30 s, 50°C for 60s and 72°C for 1 minute 30 seconds ., and a final termination at 72°C for 10 mins. And chill at 4oC.GEL (2,3). (Maestre-carballa, 2024)

3.6.3 Integrity

The integrity of the amplified gene fragment was checked on a 1% Agarose gel ran to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2 l) of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4µl of each PCR product and loaded into the wells after the 100bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 minutes visualized by

ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel. (Pupovac and Fanelli, 2023).

3.6.4 Purification of Amplified Product

After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 µl of Na acetate 3M and 240 µl of 95% ethanol were added to each about 40µl PCR amplified product in a new sterile 1.5 µl tube eppendorf, mix thoroughly by vortexing and keep at -20°C for at least 30 min. Centrifugation for 10 min at 13000 g and 4°C followed by removal of supernatant (invert tube on trash once) after which the pellet were washed by adding 150 µl of 70% ethanol and mix then centrifuge for 15 min at 7500 g and 4°C. Again remove all supernatant (invert tube on trash) and invert tube on paper tissue and let it dry in the fume hood at room temperature for 10-15 min. then resuspend with 20 µl of sterile distilled water and kept in -20oC prior to sequencing. The purified fragment was checked on a 1.5% Agarose gel ran on a voltage of 110V for about 1hr as previous, to confirm the presence of the purified product and quantified using a nanodrop of model 2000 from thermo scientific. (Skoog et al., 2017).

3.6.5 Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA 6 were used for all genetic analysis. (Maxam and Gilbert 1977)

3.7 Determination of Phenotypic Virulence Characteristics of Test Organisms

The phenotypic virulence characteristics of the test organisms, *Escherichia coli* and *Klebsiella* spp., were determined using standard microbiological methods. The tests performed include., hemolysin production, gelatinase activity, and biofilm formation. These tests were used to assess the pathogenic potential of the isolates by evaluating their ability to produce extracellular enzymes and form biofilms.

3.7.1 Gelatinase Activity

Gelatinase activity was tested using nutrient gelatin medium. Each bacterial isolate was inoculated into sterile gelatin tubes and incubated at 37°C for 24–48 hours. The tubes were then refrigerated at 4°C for 30 minutes. Liquefaction of the medium after cooling indicated a positive gelatinase reaction, confirming the organism's ability to hydrolyze gelatin into smaller peptides and amino acids. This enzyme activity contributes to tissue degradation and bacterial invasiveness (Khaled *et al.,* 2024)

3.7.2 Hemolysin Production

Hemolysin production was determined by streaking each bacterial isolate on 5% human blood agar plates. The plates were incubated at 37°C for 24 hours. After incubation, the plates were examined for zones of hemolysis surrounding the colonies. The presence of a clear (β-hemolysis) or greenish (α-hemolysis) zone around the colonies indicated positive hemolysin production, showing the organism's ability to lyse red blood cells and release hemoglobin. (Majid *et al.,* 2022).

3.7.3 Biofilm Formation

Biofilm formation was determined using the tube adherence method. The isolates were inoculated into tryptic soy broth supplemented with 1% glucose and incubated at 37°C for 24 hours without agitation. After incubation, the broth was gently decanted, and the tubes were washed with phosphate-buffered saline to remove unattached cells. The adherent cells were stained with 0.1% crystal violet, rinsed with distilled water, and air-dried. The formation of a visible violet film on the walls and bottom of the tube indicated biofilm production, whereas the absence of a film denoted a negative result. Biofilm formation enhances bacterial resistance to antimicrobial agents and host immune responses, increasing their virulence potential (Qi *et al.,* 2022).

3.8 Microbiological Analysis

3.8.1 Preparation of Agar

Four different agar media were used in this study *Salmonella–Shigella* Agar (SSA), Muller Hinton Agar (MHA), Eosin-methylene blue Agar (EMB) and Nutrient Agar (NA) each serving specific microbiological purposes.

3.8.2 Preparation of *Salmonella–Shigella* Agar (SSA),

According to manufacturer's instruction, 63 g of SSA powder was dissolved in 1000 mL of distilled water and gently heated to ensure complete dissolution. The prepared medium was sterilized by autoclaving at 121°C for 15 minutes. After sterilization, the molten agar was allowed to cool to about 45–50°C and poured aseptically into sterile Petri dishes, then left to

solidify. SSA was used mainly for the selective isolation and differentiation of enteric bacteria **such as Escherichia coli and Klebsiella spp.**

3.8.3 Preparation of Muller Hinton Agar (MHA),

38 g of MHA powder was suspended in 1000 mL of distilled water, heated with gentle swirling until completely dissolved, and then sterilized by autoclaving at 121°C for 15 minutes. The medium was allowed to cool and poured aseptically into sterile Petri dishes. MHA was specifically used for antimicrobial susceptibility testing of bacterial isolates.

3.8.4 Preparation of Nutrient Agar (NA),

28 g of Nutrient Agar powder was dissolved in 1000 mL of distilled water, mixed thoroughly, and sterilized by autoclaving at 121°C for 15 minutes. The sterilized medium was cooled to about 45°C before being poured into sterile Petri dishes and allowed to solidify. Nutrient Agar served as a general-purpose growth medium for the isolation and maintenance of pure bacterial cultures.

3.8.5 Preparation of Eosin-Methylene Blue Agar (EMB),

36g of EMB powder was suspended into 1000ml of distilled water, mixed thoroughly and sterilized by autoclaving 121°C for 15 minutes. The sterilized medium was cooled down before being poured into sterile Petri dishes (about 20ml per plate) and allowed to solidify. It is both a selective and differential medium, it inhibits Gram positive bacteria (due to eosin and methylene blue dyes).It differentiates lactose fermenters(like *E. coli*., produce acid which reacts with dyes to form dark purple colonies with a green metallic sheen) and non lactose fermenters like *salmonella* or *shigella* which produce colorless or transparent colonies.

3.8.6 Preparation Of Simmon Citrate Agar(SCA),

According to manufacturer instructions, 24.28g of SCA powder was dissolved in 1000ml of distilled water and gently heated to ensure complete dissolution. The prepared medium was sterilized by autoclaving at 121°C for 25 minutes. After sterilization the agar was allowed to cool down and then poured asceptically into sterile Petri dishes then left to solidify. It is a differential medium used to identify enteric bacteria, For the principle of the Test if it show growth with blue color it shows positive, whereas when it remains green (i.e No growth) it means negative.

3.8.7 Preparation of Tryptic Soy Broth (TSB)

30 g of Tryptic Soy Broth powder was dissolved in 1000 mL of distilled water and mixed thoroughly to ensure complete dissolution. The broth was dispensed into test tubes or flasks and sterilized by autoclaving at 121°C for 15 minutes. TSB was used as an enrichment and maintenance medium to support the growth and propagation of the bacterial isolates prior to biochemical or molecular analyses.

3.9 Enumeration And Isolation Of Bacteria From Feed Samples

Enumeration and isolation of bacteria from the food samples were carried out using the pour plate and streak plate techniques. One gram of each food sample was aseptically weighed and homogenized in 9 mL of sterile normal saline to obtain a 10^{-1} dilution. Serial dilutions were prepared up to $10^{-}4$ to reduce the bacterial concentration for proper colony isolation.

From each dilution, 0.5 mL aliquots were transferred into sterile Petri dishes and overlaid with molten Nutrient Agar, Muller Hinton Agar, Eosin-methylene blue Agar and *Salmonella–Shigella*

Agar (cooled to about 45°C). The plates were gently swirled for even distribution and allowed to solidify before incubation at 37°C for 24 hours under aerobic conditions.

To obtain pure cultures, the samples were subcultured three consecutive times on fresh Nutrient Agar plates using the streak plate method until distinct, well-isolated colonies were obtained(Charis *et al .,*2021).

3.10 Identification of Bacterial Isolates

The bacterial isolates obtained after purification were identified based on their morphological and biochemical characteristics using standard microbiological techniques.

3.10.1Morphological Test

3.10.2 Gram Staining,

Smears of the bacterial isolates were prepared and heat-fixed on clean, grease-free slides. The smears were stained for one minute with crystal violet, rinsed with distilled water, and then flooded with Gram's iodine solution for one minute. After washing with distilled water, the smears were decolorized with 95% alcohol for 30 seconds, rinsed again, and counterstained with safranin for one minute. The slides were finally washed with distilled water, air-dried, and observed under oil immersion (×100 objective) using a compound light microscope. The Gram reaction and cell morphology (shape and arrangement) were recorded (Christain *et al .,*2024).

3.10.3 Biochemical Tests

3.10.3.1 Catalase Test,

This test detects the presence of the enzyme catalase, which breaks down hydrogen peroxide into water and oxygen. A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolate smeared on a clean slide. The formation of gas bubbles indicated a positive catalase reaction.

2H_2O_2 \ 2H_2O + O_2

3.10.3.2 Citrate Utilization Test,

This test assesses the ability of an organism to utilize citrate as the sole source of carbon. Each isolate was inoculated into tubes containing Simmons citrate agar and incubated at 37°C for 24–48 hours. The development of a deep blue coloration after incubation indicated a positive citrate utilization result.

3.10.3.3 Hydrogen Sulphide (H_2S) Test,

This test detects the ability of bacteria to produce hydrogen sulphide gas (H_2S). Media containing ferrous sulphate or lead acetate as H_2S indicators and sulphur-containing substrates (such as cysteine and sodium thiosulphate) were used. The production of H_2S was indicated by the formation of a black precipitate, resulting from the reaction between H_2S and the metal salts.

3.10.3.4 Indole Test,

The indole test determines the ability of the organism to split tryptophan into indole. Each isolate was inoculated into tryptophan broth and incubated at 37°C for 18–24 hours. After incubation, 5

mL of Kovac's reagent (containing hydrochloric acid, dimethylaminobenzaldehyde, and amyl alcohol) was carefully added down the inner wall of the tube. The appearance of a bright red ring at the interface of the reagent and broth indicated a positive result for indole production.

3.10.3.5 Sugar Fermentation Test,

Each bacterial isolate was tested for its ability to ferment different sugars with the production of acid and/or gas. The medium consisted of peptone water, 1% sugar, and bromocresol purple indicator. It was dispensed into test tubes containing Durham tubes, sterilized by autoclaving at 121°C for 15 minutes, cooled, and inoculated with the test isolates. After 24 hours of incubation at 37°C, acid production was indicated by a color change from purple to yellow, while gas production was shown by displacement of the Durham tube.The sugars tested included lactose, sucrose, glucose, fructose, maltose, starch, and sorbitol.

3.10.3.6 Oxidase Test,

This test asess the ability of an organism to produce the enzyme cytochrome C oxidase. The test uses a reagent such as tetramethyl-p-phenylenediamine dihydrochloride (oxidase reagent), if the enzyme is present, the reagent is oxidized and changes color from colorless to dark purple. It is carried out by placing a piece of filter paper on a clean surface then Add a few drops of oxidase reagent using a sterile loop, pick a small amount of the bacterial colony and smear it on the paper. Observe for color change within 10-30 seconds.

3.11 Preparation of Inoculum of Food Borne Pathogens

Bacteria isolates of were grown separately in nutrient broth and incubated at 37°C for 18 hours. The bacterial suspension was standardized using a spectrophotometer to achieve a concentration

equivalent. Appropriate dilutions were made to achieve the desired infective dose for oral administration to the rats. (Jovana *et al.,* 2022).

3.12 Infection of Rats with Food-borne Pathogen

The infection of the experimental rats was carried out after the acclimatization period using an oral gavage technique. Each rat group was infected with the respective food-borne pathogens (*Escherichia coli* and *Klebsiella pneumoniae*) through oral administration using a sterile syringe fitted with a gavage needle to ensure direct delivery into the stomach. The standard innoculum volumes used were 0.06 ml and 0.1 ml, respectively, for the two concentrations designed per bacterial species. However, the exact innoculum volume administered to each group was adjusted based on the mean body weight of the rats in that group. The calculation was performed using the dilution formula $C_1V_1 = C_2V_2$, where C_1 represented the mean weight of the rats, V_1 represented the standard innoculum volume for that group, C_2 was the adjusted concentration, and V_2 was the corresponding final volume administered. This ensured that the bacterial load was normalized to the body weight of the animals. All infections were performed under aseptic conditions, and each rat was observed immediately after administration to ensure proper swallowing and to minimize stress or regurgitation (Katarzyna *et al.,* 2023).

3.13 Monitoring of Experimental Animals For Clinical Signs And Behavioral Pattern

After infection with the food-borne pathogens, all experimental rats were carefully monitored throughout the study period for any observable clinical signs and changes in behavioral patterns. Monitoring was done daily, both in the morning and evening, to evaluate the general health

status and possible pathological effects of *Escherichia coli* and *Klebsiella pnuemoniae* infection. The parameters observed included feeding habits, water intake, physical activity, posture, fur appearance, stool consistency, and signs of lethargy or distress. Particular attention was paid to loss of appetite, diarrhea, weight reduction, abnormal respiration, and dullness, which were taken as possible indicators of systemic infection or organ impairment.

The rats were also observed for mortality or abnormal reactions following oral administration of the innoculum. All observations were documented promptly in a monitoring logbook. Humane care was maintained throughout the experiment, and any animal showing severe distress or critical illness was handled in accordance with ethical laboratory animal care guidelines to minimize suffering (Margaux *et al.,* 2024).

3.14 Sacrifice and Organ Harvesting

At the end of each experimental week, selected rats from each group were humanely sacrificed for tissue and blood collection. The sacrifice was performed using chloroform inhalation to induce deep anesthesia, ensuring minimal pain or distress to the animals. Once immobilized, each rat was dissected aseptically on a clean dissection board using sterilized surgical instruments. The blood was collected carefully from the heart(while it was still beating faintly) using a sterile syringe after it was dissected.

Tissues was gently excised, rinsed in sterile normal saline to remove traces of blood, and blotted dry with sterile filter paper. The tissues were then placed in formal saline immediately after harvesting to preserve their tissue integrity and prevent autolysis. Each sample container was properly labeled according to the group and collection period before being transported to the

Histopathology Department of the University of Benin Teaching Hospital (UBTH) for processing and microscopic examination (Adetunji *et al.,* 2024).

3.17 Hematological Examination

Blood samples were collected from the experimental animals into EDTA tubes to prevent clotting. The samples were analyzed using an automated hematology analyzer to determine parameters such as red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (Hb), packed cell volume (PCV), and differential leukocyte counts. These parameters were used to assess the physiological and immune responses of the Wistar rats.

Variations in hematological values between the infected and control groups indicated the effect of *Escherichia coli* and *Klebsiella* spp. infections on blood health and immune function. (Lombardi *et al.,* 2020).

3.18 Determination of oxidative stress markers

The determination of antioxidants and oxidative stress markersninvolved using spectrophotometric methods on tissue homogenates to quantify the cellular imbalance between defense mechanisms and damage caused by the food-borne pathogens (Gkatsoudis et al., 2022). This comprehensive analysis focused on measuring the activity of three key antioxidant enzymes Superoxide Dismutase (SOD) and Catalase (CAT), which neutralize toxic oxygen radicals, and Glutathione Peroxidase (GPx), which detoxifies hydroperoxides alongside quantifying the end-product of oxidative destruction, Malondialdehyde (MDA). Since MDA is a stable marker of lipid peroxidation (Del Rio et al., 2005), its elevated presence, concurrent with changes in enzyme activity, provided direct evidence that the

bacterial infection triggered an overwhelming oxidative stress cascade in the host, leading to cellular membrane damage that was reflected in the clinical and hematological findings (Halliwell and Gutteridge, 2015).

3.19 Oxidative Stress Examination

Tissue homogenates were analyzed to evaluate oxidative stress biomarkers. Levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) were measured using spectrophotometric methods. Increased MDA levels with decreased antioxidant enzyme activities indicated enhanced lipid peroxidation and oxidative stress in infected rats. These results demonstrated that *E. coli* and *Klebsiella* infections disrupted the antioxidant defense system, leading to oxidative damage in tissues. (Gkatsoudis *et al.,* 2022).

CHAPTER FOUR

RESULT

Table 4.1 shows the molecular identification of test organisms obtained from street-vended foods. The agarose gel electrophoresis showing the positive amplification of the 16S regions amplified from the selected bacteria samples is shown in figure 1. The organisms used in this study were identified as *Escherichia coli* (ascension no., PX395408) and *Klebsiella pneumoniae* (ascension no., PX395409).

The phenotypic virulence characteristics of the test organisms is showed in Table 4.2. *E. coli* exhibited β-hemolytic activity, was positive for biofilm formation, and negative for gelatinase production, while *K. pnuemoniae* showed γ-hemolytic activity, was negative for both biofilm formation and gelatinase production.

Table 4.3 shows the total heterotrophic bacteria count (THBC) of feed samples used in this study, which ranged from 5.35±0.07 to 6.90±1.56×105 Cfu/g. The cultural, morphological and biochemical characteristics of bacteria isolated from feed samples is shown in table 4.4. Bacteria isolates identified include *Citrobacter* sp1, *Citrobacter* sp2, *Klebsiella* sp1, *Klebsiella* sp2, *Klebsiella* sp3.

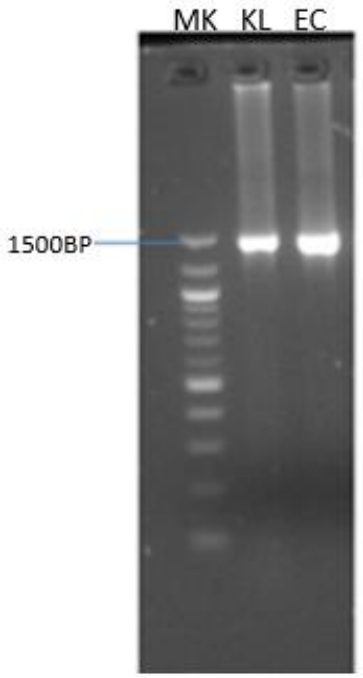
The mean weight (g) of wistar rats before and after infection with food borne pathogens is shown in table 4.5. The initial weight of the rats in week one ranged from 169.00 ± 13.90g (X1) to 175.00 ± 10.40g (W2), while the control group weighed 170.61 ± 4.90g. However, after infection, the mean weight of the rats ran±ged from 127.29 ± 9.75g to 189.35 ± 12.52g (control) and 125.76 ± 11.95g to 145.02 ± 28.94g for the experimental groups in weeks 2 to 4.

Table 4.6 shows the hematological parameters of rats infected with food borne pathogens isolated from street vended foods. The control samples fell within the range of all parameters tested expect the WBC (white blood cells) and RBC (Red blood cells) however, the WBC count of the control group was higher than the experimental groups. Rats in group W1(infected with 0.06ml E coli) recorded higher values of RBC and HGB than the control While the PLT counts of rats in group X1(0.06ml K. pneumonia) and X2(0.1ml K. pneumonia) significantly increased compared to the control. Notably PLT count of rats in group X2 (1315±546) exceeded the range of 100-500.There was significant difference in the MCV as MCH values($p<0.05$) in the infected rats compared to the control. However there were no significant difference in the WBC, RBC, HGB, HCT, MCHC and PLT values of the rats($p>0.05$).

Results of the oxidative stress and antioxidant markers is shown in table 4.7. The *K. pnuemoniae* infected rats (X_1 and X_2) induced oxidative damage reflected by a significant elevation in the SOD and MDA levels while CAT and GPx levels were significantly higher in all infected groups compared to the control samples.

TABLE 4.1, Molecular identification of Test organism from street vended foods

Sample id	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession no.
EC	Escherichia coli	1714	1714	99%	0	100.00%	PX39508
KL	*Klebsiella pneumoniae*	1722	1722	99%	0	100.00%	PX395409



KEY, MK, Molecular Marker

KL, *Klebsiella pnuemoniae*

EC*, Escherichia coli*

Figure 4.1, Agarose Gel electrophoresis showing the positive amplification of the 16S regions amplified from test organisms.

Table 4.2, Phenotypic Virulence Characteristics of Test organisms obtained from Street vended foods

Isolates	Hemoglobin production	Biofilm production	Gelatinase activity
Escherichia coli	β	+	-
Klebsiella pneumoniae	γ	-	-

KEY,

+ = Positive

-= Negative

Table 4.3, Total heterotrophic bacteria count (THBC) of feed sample

Samples	THBC (cfu/g)
A	$3.35 \pm 0.07 \times 10^5$
B	$6.9 \pm 1.56 \times 10^5$

Table 4.4, Cultural, morphological and biochemical characteristics of bacteria isolate from feed

Characteristics	1	2	3	4	5
Elevation	Low	Low	Raised	Raised	Flat
Margin	Future	Future	Future	Future	Undulate
Colony Colour	Cream	Greyish white	Mucoid	Creamy white	Cream
Colony Shape	Circular	Circular	Circular		Irregular
Colony Size	Medium	Medium	Large	Large	Large
Gram Stain	–	–	–	–	–
Cell Type	Rod	Rod	Rod	Rod	Rod
Arrangement	Disperse	Disperse	Disperse	Disperse	Single
KOH String Test	+	+	+	+	+
Catalase	+	+	+	+	+
Indole	+	+	-	-	-
Citrate	–	–	+	+	+
Oxidase	–	–	–	–	–
Glucose	+	+	+	+	+
Sucrose	–	–	+	+	+
Lactose	+	+	+	+	+
Maltose	+	+	+	+	+
Starch	–	–	–	–	–
Sorbitol	+	+	+	+	+
Fructose	+	+	+	+	+
Gas Formation	+	+	+	+	+
H_2S Formation	–	–	–	–	–
Growth on EMB	Blue-black bulls' eye with GMS	Blue-black bulls' eye with GMS	Pink mucoid colony	Pink mucoid colony	Pink mucoid colony
Identity	*Citrobacter* Sp[1]	*Citrobacter* Sp[2]	*Klebsiella* Sp[1]	*Klebsiella* sp[2]	*Klebsiella* sp[3]

TABLE 4.5 Mean weight of rats(g) before and after infection (mean±SD)

Weeks	Control	W_1	W_2	X_1	X_2
1	170.61±4.90	172.72±14.76	175.01±10.42	169.40±13.86	170.11±4.98
2	127.29±9.75	135.08±22.87	135.99±8.58	125.76±11.95	129.59±12.57
3	126.93±13.05	137.95±21.97	137.53±8.83	131.58±13.76	129.03±15.17
4	189.35±12.52	145.02±28.94	141.05±7.95	135.25±14.89	123.63±14.24

KEY, W_1 = Rats infected with 0.06ml *E. coli*, **W2 =** Rats infected 0.1ml *E. coli*, **X1 =** Rats infected 0.06ml *K. pnuemoniae*, **X2 =** Rats infected 0.1ml *K. pnuemoniae*

Table 4.6, Hematological parameters of rats infected with food borne pathogens isolated from street vended foods

Parameter	Range	Control	X1	X2	W1	W2	P-value
WBC ($\times 10^9$/L)	4-10	12.10±0.52	6.33±2.51	5.30±3.53	6.73±0.70	5.37±0.62	P>0.05
RBC ($\times 10^{12}$/L)	3.50-5.50	6.31±0.71	6.15±0.66	5.95±1.36	7.57±4.80	5.13±2.81	P>0.05
HGB (g/dL)	11.0-16.0	12.40±0.52	11.87±3.13	10.23±4.30	12.47±0.55	10.07±3.53	P>0.05
HCT (%)	36.0-48.0	39.03±2.41	37.53±4.56	37.23±9.05	29.49±15.34	33.27±7.07	P>0.05
MCV (fL)	80.0-99.0	61.63±2.56	61.13±3.69	62.50±3.01	59.00±7.01	61.70±7.80	P<0.05
MCH (pg)	26.0-32.0	19.83±2.34	19.07±3.44	16.63±4.58	18.63±2.35	18.37±4.82	P<0.05
MCHC (g/dL)	32.0-36.0	32.03±2.49	31.50±4.85	26.53±6.23	31.77±4.12	29.57±5.29	P>0.05
PLT ($\times 10^9$/L)	100-500	348.67±51.81	440.33±110.65	1315.00±1446.30	255.33±198.00	340.00±294.96	P>0.05

KEY, **W_1** = Rats infected with 0.06ml *E. coli*, **W2** = Rats infected 0.1ml *E. coli*, **X1** = Rats infected 0.06ml *K. pnuemoniae*, **X2** = Rats infected 0.1ml *K. pnuemoniae* ;**WBC**=White blood cells, **RBC**=Red blood cells, **HGB=**Hemoglobin, **HCT=**Hematocrit, **MCV=**Mean corpuscular volume,**MCH**=Mean corpuscular hemoglobin, **MCHC**= Mean corpuscular hemoglobin concentration, **PLT=**Platelets

4.7, Results of the oxidative stress and anti oxidant markers of wistar rats infected with food borne pathogens isolated from street vended foods

Parameters	SOD (U/g Prot)	CAT (U/g Prot)	GPx (U/g Prot)	MDA (Mol/g Prot)
CONTROL	0.514±0.284	0.2704±0.055	0.359±0.023	0.120±0.030
W1	0.303±0.135	0.3644±0.246	0.426±0.270	0.110±0.044
W2	0.413±0.040	0.3274±0.184	0.3870±0.188	0.120±0.040
X1	0.719±0.005	0.3514±0.057	0.479±0.022	0.140±0.005
X2	0.900±0.817	0.4824±0.257	0.576±0.221	0.170±0.059

Key, W_1 = Rats infected with 0.06ml *E. coli*,**W2 =** Rats infected 0.1ml *E. coli*,**X1 =** Rats infected 0.06ml *K. pnuemoniae*, **X2 =** Rats infected 0.1ml *K. pnuemoniae*

Superoxide Dismutase Activity (SOD),Catalase Activity (CAT),Gluthathione Peroxidase (GPx),Malondialdehyde (MDA)

CHAPTER FIVE

5.1 DISCUSSION

The molecular identification of test organisms using 16S rRNA gene sequencing confirmed the presence of *Escherichia coli* (accession no. PX395408) and *Klebsiella pneumoniae* (accession no. PX395409) isolated from street-vended foods. Both organisms showed 100% identity match with maximum scores of 1714 and 1722 respectively, and 99% query coverage, indicating high confidence in their taxonomic classification. The successful amplification of the 16S regions as shown in Figure 1 validates the molecular approach used for bacterial identification in this study. According to a systematic review by (Mengistu *et al*., 2022) *E. coli* and *Klebsiella* species constituted 23.8% and 9.1% respectively of bacterial contamination in ready-to-eat foods from developing countries. The presence of these organisms in street-vended foods is particularly concerning given their well-established pathogenic potential and their role as indicators of fecal contamination. However, contrasting findings were reported by (Okiki *et al*., 2019) in a study conducted across four major towns in Ethiopia, where Staphylococcus aureus (27.2%) was the most abundant contaminant, while *E. coli* was detected in only 19% of samples. The diversity of bacterial contaminants in their samples, may reflect regional differences in food handling practices, environmental conditions, or vendor training levels. *Escherichia coli* is commonly used as an indicator organism for fecal contamination in food and water, and its presence suggests inadequate hygiene practices during food preparation and handling. Similarly, *K. pnuemoniae*, while naturally occurring in the environment, is increasingly recognized as an opportunistic pathogen capable of causing severe infections, particularly hypervirulent strains that can affect immunocompetent individuals.

The phenotypic virulence characteristics of the isolates, revealed pathogenic traits in both test organisms. *Escherichia coli* exhibited β-hemolytic activity, positive biofilm formation, and negative gelatinase production, while *Klebsiella pneumoniae* demonstrated γ-hemolytic activity and negative for biofilm and gelatinase production. Similarly, (Adhikari *et al.,* 2023) isolated *E. coli* from street-vended foods sold at food stalls in Bharatpur, Nepal, displaying comparable pathogenic traits. In their findings, 41.67% of the isolates were biofilm producers, among which *E. coli* constituted 46.38% of the biofilm-producing organisms. These virulence factors play critical roles in the pathogenicity and survival of these organisms in both food matrices and host tissues. The hemolytic activity observed in both organisms is a significant virulence determinant. The β-hemolytic activity of *E. coli* indicates complete hemolysis and is associated with the production of α-hemolysin (HlyA), a pore-forming toxin that contributes to tissue damage and disease severity. This toxin has been implicated in various extraintestinal infections and can cause direct cytotoxic effects on endothelial cells, epithelial cells, and immune cells. The positive biofilm formation observed in *E. coli* in this study, is particularly concerning from both food safety and clinical perspectives. In the context of food safety, biofilm formation enhances bacterial persistence on food contact surfaces, equipment, and utensils, making them highly resistant to cleaning and disinfection procedures. From a clinical standpoint, biofilm-producing bacteria exhibit enhanced virulence and are more resistant to host immune defenses and antimicrobial agents. Bacteria within biofilms can be 10 to 1000 times more resistant to antibiotics compared to their planktonic counterparts.

The total heterotrophic bacterial count (THBC) of the feed samples ranged from 5.35 ± 0.07 (Sample A) to 6.90 ± 1.56 × 10^5 CFU/g (Sample B). Similarly, findings by (Matthew *et al.,* 2017) revealed high microbial loads in poultry feed, with bacterial counts of 5.4 × 10^5 CFU/g in broiler

feed and fungal counts as high as 8.1×10^5 CFU/g, indicating inadequate microbial control. These values represent the microbial load in the pelleted feed used for the wistar rats in this study. According to the International Commission on Microbiological Specifications for Foods (ICMSF, 1986), acceptable aerobic plate counts for feed should generally be below 10^5 CFU/g, as higher counts may indicate contamination, poor storage, or suboptimal processing hygiene. The elevated counts observed in this study suggest possible post-processing contamination or microbial proliferation during storage, which can occur when feed is exposed to humid conditions or stored in inadequately sealed containers. The presence of high bacterial counts in animal feed is significant because it can influence the gut microbiota of experimental animals, potentially altering physiological responses and compromising experimental validity. Moreover, microbial contamination of feed may serve as a reservoir for opportunistic pathogens, posing systemic risk. Therefore, proper storage under dry, ventilated conditions and periodic microbial assessment of feed pellets are essential to minimize contamination risks and maintain the health of laboratory animals used in experimental studies.

The cultural, morphological, and biochemical characterization of bacteria isolated from feed samples revealed the presence of five distinct isolates identified as *Citrobacter* sp^1, *Citrobacter* sp^2, *Klebsiella* sp^1, *Klebsiella* sp^2, and *Klebsiella* sp^3. All isolates were Gram-negative rods with similar cellular arrangements but exhibited variations in colony characteristics and biochemical properties. The isolates displayed diverse colony morphologies, with Citrobacter species showing low convex elevation with cream to greyish-white coloration, while *Klebsiella* species exhibited raised elevation with mucoid, creamy-white to creamy coloration. The mucoid appearance of *Klebsiella* species colonies is characteristic of this genus and is attributed to the production of abundant capsular polysaccharides, which serve as important virulence factors by

protecting the bacteria from phagocytosis and complement-mediated killing. All isolates were positive for catalase, citrate utilization, glucose fermentation, sucrose fermentation, lactose fermentation, and gas formation, which are characteristic features of Enterobacteriaceae. The presence of Enterobacteriaceae in the feed samples suggests poor hygiene practices during the feed preparation, storage and handling process. Both *Citrobacter* and *Klebsiella* species are opportunistic pathogens belonging to the Enterobacteriaceae family and are frequently associated with nosocomial infections and community-acquired infections. The isolation of multiple *Klebsiella* species strains from feed samples is particularly noteworthy given the emergence of hypervirulent *K. pnuemoniae* strains that can cause severe infections even in healthy individuals. Recent genomic studies have revealed concerning trends in *Klebsiella* epidemiology. Findings by Wan *et al.,* 2025 from a Chinese study analyzing *K. pnuemoniae* isolates from pig feeds revealed high genetic diversity and multiple sequence types, suggesting significant zoonotic potential. These findings emphasize that *Klebsiella* strains may represent reservoirs of potentially hypervirulent or multidrug-resistant lineages that could pose significant public health threats.

The changes in mean weight of Wistar rats before and after infection with *E. coli* and *K. pnuemoniae* provide important insights into the systemic impact of these food-borne pathogens. The initial weights in week 1 for the control was 170.61 ± 4.90g while the initial weights for the experimental rats ranged from 169.40 ± 13.86g (X_1) to 175.0n1 ± 10.42g (W_2), indicating adequate randomization and homogeneity among the experimental groups at baseline. The changes in body weight following infection, at week 2 showed that there was a decrease in body weight in all groups (including the control) from week 1 to 2. Rats in the control group, experienced a very slight decrease in weight, in week 2 to 3, and subsequently gained more

weight till week 4. While rats in groups W_1, W_2 and X_1 showed an increase in weight from weeks 2 to 4. However, rats in group X_2 (0.1 *K. pnuemoniae*) recorded an almost constant weight in weeks 2-3 and loss in body weights at week 4. The general decrease in body weight observed across all groups in week 1 to 2 could be as a result of the rats adapting to the new environment and feed provided. Following adaptation, a robust increase in body weight was observed in the control group. Similarly, the rats in groups W_1, W_2 and X_1 also gained weight, which was lower compared to the control. This may have been due to the physiological state of the rats in the experimental groups following infection. Rats in group X_2 lost more body weight, probably due to the virulence of *K. pnuemoniae* strains, infection dose used and a possible co-infection following the presence of *K. pnuemoniae* in the feed. Findings by Assoni *et al.,* (2024) in a review on animal models of *K. pnuemoniae* mucosal infections noted that hypervirulent strains often prove lethal in mouse studies, with systemic dissemination leading to rapid deterioration. This decrease reflects the acute phase of infection, characterized by metabolic alterations, reduced feed intake, and increased energy expenditure associated with immune response actidurinn. During bacterial infections, pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6) are released, leading to anorexia, enhanced protein catabolism, and altered energy metabolism.

Result of hematological examination revealed that the values for the control fell within the recommended range. However, the WBC (12.01±0.52) and RBC (6.31± 0.71) counts except for the W1 group were higher than the experimental design group (5.30±5.33 to 6.73±0.70 and 5.13±2.81 to 6.15±0.66 respectively). These results, are in accordance with the work of (Sikiru *et al.,* 2024) who reported higher WBC counts (7.86±0.94) in unexposed rats(control) compared with rats exposed to Aflatoxin B1. Similarly, (Chattopadhyay *et al*., 2018) also reported higher

counts in WBC (8.07±0.127) and RBC (3.48±0.50) in untreated rats(control) compared with treated rats (7.85±0.08) and (1.90±0.47) respectively. The drop in WBC counts observed in the samples from the experimental groups may have been due to immune suppression caused by the infection. While the drop in RBC counts in infected rats may possibly be as a result of red blood cell haemolysis caused by bacterial infection (El-Demerdash, 2004). This was most evident in group W_2 (infected with 0.1ml *E*.coli) which recorded the lowest RBC count of 5.13±2.81 as *E.coli* was shown to exhibit a greater haemolytic activity from the results of the phenotypic virulence screening in this study. Hence a higher dose (0.1ml) resulted in greater effect on the RBC count. The drop in platelet counts observed mainly in rats infected with *E.coli,* in group W_1 (255.33) and W_2 (340.00) may also be as a result of anaemia caused by the infection with *E. coli* compared to the other groups. The significant difference observed in the values for the mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MHC) between the infected rats and the control (uninfected group) could have resulted from iron deficiency caused by the bacterial infection. These results are in accordance with the work of (Okiki *et al.,* 2019), who also reported a drop in WBC, RBC, PLT, MCV and MHC values in *Salmonella typhimurium* infected rats, compared to the control group.

Results from oxidative stress markers reveals the oxidative stress response and antioxidant system collapse in rats infected with foodborne pathogens, demonstrating how bacterial infection triggers a cellular war against reactive oxygen species (ROS) that ultimately leads to membrane destruction and multi-organ failure. Superoxide dismutase (SOD) activity, which represents the first line of defense by converting toxic superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2), showed dramatically divergent responses across groups, W1 (*K. pnuemoniae* 0.1 mL) exhibited the lowest activity at 0.303 ± 0.135 U/g protein, suggesting complete antioxidant exhaustion

from prolonged high-dose bacterial challenge, while paradoxically X2 (*E. coli* 0.06 mL) displayed the highest SOD activity at 0.900 ± 0.817 U/g protein a 75% increase over the control's 0.514 ± 0.284 indicating maximal compensatory upregulation as cells desperately attempt to neutralize the massive superoxide production from neutrophil respiratory burst and mitochondrial dysfunction triggered by *E. coli*'s biofilm-sustained endotoxin release.(Awapak *et al.,* 2021).However, this SOD upregulation proves futile because catalase (CAT) activity, which should convert the resulting hydrogen peroxide into harmless water and oxygen, was severely suppressed across all infected groups, with W2 (*K. pnuemoniae* 0.06 mL) showing the most dramatic collapse at 0.3274 ± 0.184 U/g protein compared to the control's 0.774 ± 0.055a devastating 58% reduction that allows H_2O_2 accumulation to create hydroxyl radicals (•OH) through Fenton chemistry with free iron released from damaged cells, representing the most destructive ROS that indiscriminately attacks all cellular components. To compensate for this catalase failure, glutathione peroxidase (GPx) activity increased across infected groups, with X2 reaching 0.576 ± 0.221 U/g protein (a 60% elevation over control's 0.359 ± 0.023), as this selenium-dependent enzyme attempts to reduce both H_2O_2 and lipid hydroperoxides using glutathione as a sacrificial electron donor, but this backup system cannot fully compensate when catalase the primary high-capacity H_2O_2 disposal system is crippled. The ultimate proof of oxidative damage appears in malondialdehyde (MDA) levels, an end-product of lipid peroxidation that directly quantifies membrane destruction, all infected groups showed elevated MDA, with X2 reaching 0.170 ± 0.059 U/g protein (a 42% increase over control's 0.120 ± 0.030), demonstrating that despite maximal SOD and GPx upregulation, the antioxidant defenses are overwhelmed, allowing ROS to attack polyunsaturated fatty acids in cell membranes, creating a chain reaction of lipid breakdown that compromises membrane integrity in red blood cells

(explaining the anemia in Table 4.6), hepatocytes, renal tubules, and vascular endothelium. The pattern reveals a critical insight, X2's simultaneous peaks in SOD (0.900), GPx (0.576), and MDA (0.170) demonstrate that lower-dose *E. coli* with biofilm capability creates chronic, sustained oxidative assault that maximally activates compensatory antioxidases while still causing the worst membrane damage, whereas W1's low SOD (0.303) with moderate MDA (0.110) suggests antioxidant exhaustion from prolonged high-dose *K. pnuemoniae* challenge that depletes enzyme reserves before catastrophic lipid peroxidation fully manifests. This oxidative stress cascade directly links to the hematological findings, MDA-damaged RBC membranes become fragile and prone to hemolysis (explaining low hemoglobin in X1), oxidative injury to bone marrow stem cells impairs hematopoiesis (explaining pancytopenia), and vascular endothelial oxidative damage triggers platelet consumption (explaining thrombocytopenia), while the energy diverted to synthesizing antioxidant enzymes and repairing oxidative damage explains the severe weight loss in Table 4.5, collectively demonstrating that foodborne Gram-negative bacteria kill not primarily through direct tissue invasion but through triggering an uncontrolled oxidative firestorm that the host's antioxidant systems despite heroic upregulation cannot extinguish, validating oxidative stress biomarkers (particularly the MDA elevation and SOD/CAT imbalance) as early harbingers of sepsis progression that could guide clinical intervention before irreversible multi-organ failure occurs.(Elbaz *et al*., 2020)

5.2 CONCLUSION

This study has demonstrated that food-borne pathogens isolated from street-vended foods can cause significant alterations in both hematological parameters and oxidative stress biomarkers in experimental animals. The findings revealed that infection with *Escherichia coli* and *Klebsiella pneumoniae* led to reduced levels of red blood cells, hemoglobin, and packed cell volume,

indicating anemia and compromised oxygen transport, while elevated white blood cell counts reflected an active immune response to infection. Furthermore, increased malondialdehyde (MDA) levels and decreased antioxidant enzyme activities (SOD, CAT, and GSH) indicated lipid peroxidation and oxidative imbalance, confirming that these pathogens induce oxidative stress and cellular damage. The severity of these effects was more pronounced in animals exposed to higher pathogen concentrations, suggesting a dose-dependent response. Overall, the study establishes a clear link between the consumption of contaminated street-vended foods and potential systemic health risks, emphasizing the urgent need for improved hygiene standards, vendor education, and strict enforcement of food safety regulations. Ensuring safe food handling, routine microbial monitoring, and public health awareness campaigns will help minimize the incidence of food-borne infections and safeguard community health.

5.3 RECOMMENDATION

5.3.1 Regular Hematological Monitoring of Exposed Populations

Street food consumption contaminated with pathogens such as Salmonella and Staphylococcus aureus can induce anemia-like conditions by reducing RBC count, hemoglobin, and packed cell volume in Wistar rats, suggesting routine blood screening for frequent street food consumers. (Afolabi *et al*., 2015).

5.3.2 Antioxidant Supplementation to Counter Oxidative Stress

Foodborne pathogens from contaminated street foods elevate malondialdehyde (MDA) levels while depleting superoxide dismutase (SOD) and catalase activity in rat models, indicating oxidative damage that may be mitigated through dietary antioxidants. (Ohimain and Ofongo 2012).

5.3.3 Strengthening Street Food Hygiene Regulations

Microbial loads found in street-vended foods have been linked to leukocytosis and elevated white blood cell counts in Wistar rats, reflecting systemic immune activation that underscores the need for stricter vendor hygiene enforcement. (Barro *et al*., 2007).

REFERENCES

. Adekunle, I. A., Ojo, S. K., and Adebayo, J. O. (2022). Thrombocytopenia in experimental *Escherichia coli* sepsis in Wistar rats. *Journal of Veterinary Medical Science***, 84**(3);345–352.

Adewoye, E. O., Adeyemo, O. A., and Salako, A. B. (2021). Hematological changes in Wistar rats infected with *Klebsiella pneumoniae*. *African Journal of Microbiology Research*, **15**(7);301–308.

Argilés, J. M., Busquets, S., Stemmler, B., and López-Soriano, F. J. (2014). Cancer cachexia, Understanding the molecular basis. *Nature Reviews Cancer,* (**14**),754–762.

Assoni, L., Ana, C., Brenda, V., (2024). Animal models of *Klebsiella pneumoniae* mucosal infections. *Frontiers in Microbiology*,**15**; 1367422

Awapak, D., Petchkongkaew, A., Sulyok, M., and Krska, R. (2021). Co-occurrence and toxicological relevance of secondary metabolites in dairy cow feed from Thailand. *Food Additives and Contaminants* **38**(6); 1013–1027

Caza, M., and Kronstad, J. W. (2013). Shared and distinct mechanisms of iron acquisition by bacterial and fungal pathogens. *Cellular Microbiology*, **15**;1496–1506.

Chattopadhyay, M., Das, D., Gupta, M. and Bagchi, G.k. (2018). Assessment of haematological and biochemical effects of acute and chronic administration of the *Stenotrophomonas*

maltophilla lysates in mice. *Journal of Microbiology Biotechnology and Food Science* **8(3)**: 936-939.

Clarridge, J. E., III. (2004). Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clinical Microbiology Reviews,* **17**;840–862.

Del Rio, D., Stewart, A. J., and Pellegrini, N. (2005). A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Free Radical Biology and Medicine,* **39**;1035–1045.

EFSA Panel on Biological Hazards (BIOHAZ). (2017). Scientific opinion on the risk for animal and public health related to the presence of *Salmonella* in feed. *EFSA Journal*,**1;**04791.

Elbaz, H. S., Hamed, M. F., Abdelhamid, F. M., and Abdalla, O. A. (2020). Effect of cefepime on hematological, immunological and oxidant/antioxidant parameters in rats experimentally infected with *E. coli* ATCC 25922. *Mansoura Veterinary Medical Journal*, **21**;36–45.

El-Demerdash, F.M. (2004). Antioxidant effect of vitamin E and selenium on lipid peroxidation, enzyme activities and biochemical parameters in rats exposed to aluminium. *Journal of Trace Elements and Medicine and Biology* **8**: 113-122

Flemming, H.-C., and Wingender, J. (2010). The biofilm matrix. *Nature Reviews Microbiology*, **8**;623–633.

Flemming, H.-C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., and Kjelleberg, S. (2016). Biofilms, An emergent form of bacterial life. *Nature Reviews Microbiology*, **14**;563–575.

Halliwell, B., and Gutteridge, J. M. C. (2015). Free radicals in biology and medicine (5th ed.). Oxford University Press.

Hotchkiss, R. S., Moldawer, L. L., Opal, S. M., Reinhart, K., Turnbull, I. R., and Vincent, J.-L. (2016). Sepsis and septic shock. *Nature Reviews Disease Primers,* **2**;16045.

Hotchkiss, R. S., Monneret, G., and Payen, D. (2013). Sepsis-induced immunosuppression, From cellular dysfunctions to immunotherapy. *Nature Reviews Immunology,* **13**(12);862–874.

Lebeaux, D., Ghigo, J.-M., and Beloin, C. (2014). Biofilm-related infections, Bridging the gap between clinical management and fundamental research. *Clinical Microbiology and Infection***, 20**;1015–1023.

Levi, M., and Scully, M. (2018). How do we manage thrombocytopenia in sepsis? Blood **131**;735–742.

Levi, M., and Ten Cate, H. (2010). Disseminated intravascular coagulation. *New England Journal of Medicine***, 341**(8); 586–592.

Mengistu, D. A., Belami, D. D., Tefera, A. A., and Asefa, Y. A. (2022). Bacteriological Quality and Public Health Risk of Ready-to-Eat Foods in Developing Countries: Systematic Review and Meta Analysis. *Microbiology Insights*, 15;11786361221113916.

Nemeth, E., Tuttle, M. S., Powelson, J., Vaughn, M. B., Donovan, A., Ward, D. M., Ganz, T., and Kaplan, J. (2004). Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* **306**: 2090–2093.

Okiki, P. A., Ahmad, M. A., and Temitayo, O. O. (2019). Anti-inflammatory actions and *Salmonella* typhimurium-bacteraemia clearance by methanol extract of Curcuma longa Linn. (Turmeric). *Malaysian Journal of Microbiology*,**15**; 24–33.

Olaniyan, M. F., and Ojedirian, O. O. (2020). Macrocytosis in chronic bacterial infection, A hematological paradox. *Journal of Hematology Research,* **8**(1); 12–19.

Olayinka, B. O., Olaniyan, M. F., and Adewole, T. A. (2019). Dose-dependent leukopenia in Escherichia coli-challenged Wistar rats. *Nigerian Journal of Physiological Sciences*, **34**(2); 115–120.

Paczo, A., Dębski, J., and Łukasiak, M. (2018). Capsule of *Klebsiella pneumoniae* as a virulence factor. *Frontiers in Microbiology***, 9;** 152.

Podschun, R., and Ullmann, U. (1998). *Klebsiella* spp. as nosocomial pathogens, Epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clinical Microbiology Reviews***,** **11**(4);589–603.

Rivera, S., Liu, L., Nemeth, E., Gabayan, V., Sorensen, O. E., and Ganz, T. (2005). Anemia of inflammation, The cytokine-hepcidin link. *Current Opinion in Hematology*, **12**(2); 107–111.

Sikiru, A. B., Egena, S. S. A., Alemede, I. C., Alabi, J. O., Makinde, J. O., and Behera, H. (2024). Effect of aflatoxin-B1 exposure on performance and hematological stress indicators in male Sprague Dawley rats. *Fountain Journal of Natural and Applied Sciences* **13**:1–14.

Seye, A., Leonard, B., Maryam, I. (2018). The impacts of decentralization on health system equity, efficiency and resilience. *A realist synthesis of the evidenceHealth policy and planning* **34** (8), 605-617

Wang, P., Huang, J., Li, Y., and Chang, R. (2016). Acute hematological collapse in sepsis, A murine model of cytokine storm. *Critical Care Medicine***, 44**(5); e387–e395.

Weiss, G., and Goodnough, L. T. (2005). Anemia of chronic disease. *New England Journal of Medicine*, **352**(10); 1011–1023.